Waters
ACQUITY UPC²
Bibliography

A compendium of references for the ACQUITY UPC² system

UltraPerformance
Convergence Chromatography

August 2018

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328. Enantioselective Formation Of Substituted 3,4-Dihydrocoumarins By A Multicatalytic One-Pot Process

2012 - Organic Letters
1. Systematic evaluation of matrix effects in supercritical fluid chromatography versus liquid chromatography coupled to mass spectrometry for biological samples
Vincent Desfontaine, Francesca Capetti, Raul Nicoli, Tiia Kuuranne, Jean-Luc Veuthey, Davy Guillarme

Keywords: Matrix effects, LC-MS, SFC-MS, Urine, Plasma

Abstract
Matrix effects (ME) is acknowledged as being one of the major drawbacks of quantitative bioanalytical methods, involving the use of liquid chromatography coupled to mass spectrometry (LC-MS). In the present study, the incidence of ME in SFC-MS/MS and LC-MS/MS in the positive mode electrospray ionization (ESI+) was systematically compared for the analysis of urine and plasma samples using two representative sets of 40 doping agents and 38 pharmaceutical compounds, respectively. Three different SFC stationary phase chemistries were employed, to highlight the importance of the column in terms of selectivity. Biological samples were prepared using two different sample treatments, including a non-selective sample clean-up procedure (dilute and shoot (DS) and protein precipitation (PP) for urine and plasma samples, respectively) and a selective sample preparation, namely solid phase extraction (SPE) for both matrices. The lower susceptibility to ME in SFC vs. reversed phase LC (RPLC) was verified in all the experiments performed on urine, and especially when a simple DS procedure was applied. Also, with the latter, the performance strongly varied according to the selected SFC stationary phase, whereas the results were quite similar with the three SFC columns, in the case of SPE clean-up. The same trend was observed with plasma samples. Indeed, with the PP procedure, the occurrence of ME was different on the three SFC columns, and only the 2-picolylamine stationary phase chemistry displayed lower incidence of ME compared to LC-MS/MS. On the contrary, when a SPE clean-up was carried out, the results were similar to the urine samples, with higher performance of SFC vs. LC and limited discrepancies between the three SFC columns. The type of ME observed in LC-MS/MS was generally a signal enhancement and an ion suppression for urine and plasma samples, respectively. In the case of SFC-MS/MS, the type of ME randomly varied according to the analyzed matrix, selected column and sample treatment.

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2. Screening of stationary phase selectivities for global lipid profiling by ultrahigh performance supercritical fluid chromatography
Said Al Hamimi, Margareta Sandahl, Marina Armeni, Charlotte Turner, Peter Spédel

Keywords: Mass spectrometry, Lipidomics, Design of experiments, Blood serum

Abstract

https://doi.org/10.1016/j.jchroma.2018.01.037
The performance of seven sub-2-μm particle packed columns (2-picolyamine, 2-PIC; charged surface hybrid fluoro-phenyl, CSH-FP; high strength silica C18 SB, HSS-C18; diethylamine, DEA; 1-aminoanthracene, 1-AA; high density diol and ethylene bridged hybrid; BEH) was examined for lipid separation in ultra-high performance supercritical fluid chromatography (UHPSFC) coupled to quadrupole time-of-flight mass spectrometry. Based on the results of the column screening a method for profiling of multiple lipid species from the major lipid classes was developed. Stationary phases containing β-hydroxy amines, i.e. 1-AA, DEA and 2-PIC, yielded strong retention and poor peak shapes of zwitterionic lipids with primary amine groups, such as phosphatidylycerines, phosphatidylethanolamines and its lyso forms. The BEH and HSS-C18 columns showed strong retention of polar and nonpolar lipids, respectively. The Diol column retained the majority of major lipid classes and also produced symmetric peaks. In addition, this column also produced the highest resolution within and between major lipid classes. An injection solvent composed of methanol:chloroform (1:2, v:v) and the addition of 20 mM ammonium formate in the mobile phase improved chromatographic separation and mass spectrometry detection in comparison to ammonium acetate or absence of additive. Finally, chromatographic and mass spectrometric parameters were optimized for the Diol column using a design of experiments approach. The separation mechanism on the Diol column depended on the lipid functionality and the length and degree of unsaturation of the acyl groups. The developed method could resolve 18 lipid classes and multiple lipids within each class, from blood serum and brain tissue in 11 min.

https://doi.org/10.1016/j.chroma.2018.03.024

3. Forensic applications of supercritical fluid chromatography – mass spectrometry
Volodymyr Pauk, Karel Lemr

Keywords: Drug of abuse, New psychoactive substance, Doping control, Food authenticity, Adulteration

Abstract
Achievements of supercritical fluid chromatography with mass spectrometric detection made in the field of forensic science during the last decade are reviewed. The main topics include analysis of traditional drugs of abuse (e.g. cannabis, methamphetamine) as well as new psychoactive substances (synthetic cannabinoids, cathinones and phenethylamines), doping agents (anabolic steroids, stimulants, diuretics, analgesics etc.) and chemical warfare agents. Control of food authenticity, detection of adulteration and identification of toxic substances in food are also pointed out. Main aspects of an analytical workflow, such as sample preparation, separation and detection are discussed. A special attention is paid to the performance characteristics and validation parameters of supercritical fluid chromatography-mass spectrometric methods in comparison with other separation techniques.
4. **Comparison between liquid chromatography and supercritical fluid chromatography coupled to mass spectrometry for beta-agonists screening in feeding stuff**

L. Herpin, E. Bichon, L. Rambaud, F. Monteau, B. Le Bizec

Keywords: β-agonists, UHPLC-MS/MS, SFC-MS/MS, Feeding stuff, Growth promoters monitoring

**Abstract**

β-agonistic drugs have been forbidden as growth promoters in rearing animals in Europe since the late 1980s (Dir 96/22/EC). Specific and sensitive analytical methods based on UHPLC-MS/MS allow to monitor a large set of these substances. However, optimal performances are not observed for all the target analytes, especially for those exhibiting the highest polarities. We developed an SFC-MS/MS approach to cover the huge elution window of β-agonists, from the most polar which are usually eluted in the void volume when using reversed phase chromatography in conventional HPLC to the most apolar ones. The objective was to reach performances in accordance with the European Union recommended level in feeding stuff, i.e. 50 μg kg\(^{-1}\). LC/MS and SFC/MS performances were thoroughly compared in terms of analytical validation data (linearity, selectivity, recovery rates, reproducibility, compounds identification, trueness, decision limit (CC₀) and detection capability (CCβ)) for 6 β-agonistic drugs, namely bromobuterol, clenbuterol, isoxsuprine, ractopamine, salbutamol and zilpaterol. As a result, the SFC approach appeared complementary to the LC one because the elution order of compounds was totally different from the one obtained with a classical C18 stationary phase. Moreover, the UPLC-MS/MS approach gave a better response linearity and more accurate values, whereas SFC-MS/MS provided greater data for identification purposes, reproducibility and sensitivity. Both analytical approaches enabled the detection of targeted β-agonists at a lower concentration than the recommended one (50 μg kg\(^{-1}\)).
5. Analysis of multiple vitamin D metabolites by ultra-performance supercritical fluid chromatography-tandem mass spectrometry (UPSFC-MS/MS)

Carl Jenkinson, Angela Taylor, Karl-Heinz Storbeck, Martin Hewison

Abstract

In recent years, increased interest in the human health benefits of vitamin D has led to demand for improved analysis of patient vitamin D ‘status’. Studies to date have focused primarily on a single vitamin D metabolite, 25-hydroxyvitamin D, despite the existence of a broad range of vitamin D metabolites, referred to as the vitamin D metabolome. This study reports on the development of a rapid UPSFC-MS/MS method for the analysis of nine vitamin D metabolites in human serum. Optimum separation was obtained with a Lux-Cellulose chiral column. We observed an orthogonal elution order when compared with ultra-high performance liquid chromatography (UHPLC). The order of elution was reversed based on hydroxyl- group number, however elution order did not differ between isomeric changes in hydroxyl- group position or epimers. Although UPSFC yielded superior resolution and selectivity over previously developed UHPLC-MS/MS methods, improvements in sensitivity could not be achieved owing to the lower injection volume required for UPSFC relative to UHPLC. Method validation was performed on the developed UPSFC-MS/MS method and found to be within acceptable limits. Applying the method to the analysis of human serum samples showed a significant correlation with serum concentrations of metabolites measured by UHPLC-MS/MS (25OHD3 r = 0.997, P < 0.001, and 3-epi-25OHD3 r = 0.996, P ≤ 0.001). These data indicate that UPSFC provides an efficient analytical platform for rapid analysis of multiple vitamin D metabolites from serum.
6. **Novel and rare prenyllipids – Occurrence and biological activity**

Renata Szymańska, Jerzy Kruk

2018 – Plant Physiology and Biochemistry

**Keywords:** Prenyllipids, Tocochromanols, Plastoquinones, Ubiquinones, Vitamin E, Antioxidants

**Abstract**

The data presented indicate that there is a variety of unique prenyllipids, often of very limited taxonomic distribution, whose origin, biosynthesis, metabolism and biological function deserves to be elucidated. These compounds include tocoenols, tocochromanol esters, tocochromanol acids, plastoquinones and ubiquinones. Additionally, based on the available data, it can be assumed that there are still unrecognized prenyllipids, like prenylquinols fatty acid esters of the hydroquinone ring, including prenylquinol phosphates, and others, whose biological function might be of great importance. Our knowledge of these compounds is not only important from the scientific point of view, but may also be of practical significance to medicine, pharmacy or cosmetics.
7. Continuous multicomponent quantification during supercritical fluid extraction applied to microalgae using in-line UV/Vis absorption spectroscopy and on-line evaporative light scattering detection
Victor Abrahamsson, Firas Jumaah, Charlotta Turner

Keywords: Carotenoids, Chromatography, Microalgae, SFC, SFE, Analytical method

Abstract
A quantitative methodology based on in-line UV/Vis absorption spectroscopy and on-line evaporative light scattering detection for supercritical fluid extraction is proposed. The method was applied to the extraction of carotenoids, chlorophyll A, ergosterol and total lipids from microalgae. One regression technique and two curve resolution techniques were applied on the absorption spectroscopy data and evaluated, namely classical least squares, multivariate curve resolution by alternating least squares and parallel factor analysis (PARAFAC2). The two former both generated useful models, furthermore multivariate curve resolution also successfully enabled estimation of both spectra and concentration profiles of the analytes. The integrated extraction profiles of each analyte were compared with analysis of the collected fractions using reference analysis methods. Precision, in regards to quantification of the analytes in the eluent, was better using in-line measurements compared to off-line measurements by UV/Vis absorption spectroscopy, supercritical fluid chromatography with mass spectrometry and liquid chromatography with UV/Vis detection.
8. Interest of achiral-achiral tandem columns for impurity profiling of synthetic drugs with supercritical fluid chromatography

Caroline West, lise Lemasson, Sophie Bertin, Philippe Hennig, Eric Lesellier

Keywords: High-resolution separations, Impurity profiling, Orthogonal methods, Performance comparison, Pharmaceutical ingredients, Tandem columns

Abstract

To achieve the most complete impurity profiling of synthetic drugs with a single chromatographic technique, high resolution is required, which may be gained with a combination of high efficiency and versatile selectivity, allowing to separate most similar analytes. Compared to a single-column chromatographic method, coupling complementary stationary phases promises both an increase in efficiency and an increase in selectivity possibilities. With supercritical fluid chromatography (SFC), the use of long columns is facilitated by the low viscosity of the mobile phase. In this paper, we investigate the interest of coupling two achiral stationary phases (Acquity UPC² HSS C18 SB and Nucleoshell HILIC) that were previously observed to have excellent complementarity in SFC to carry out impurity profiling on 25 individual drug substances containing varied numbers and amounts of impurities. The single-column gradient methods are compared to tandem-column gradient methods with the two possible ordering of columns (C18 phase in first or second position) based on selectivity, peak capacity, sensitivity, UV-estimated purity of the active pharmaceutical ingredient and number of impurities detected with UV-estimated concentration >0.04%. It appears that it could be more beneficial to have two columns coupled in a single analysis than two consecutive methods with the single columns. The overall analysis time are nearly the same, but with more informative chromatograms in about 35% cases.
9. **Inefficient UGT-conjugation of adrenal 11β-hydroxyandrostenedione metabolites highlights C11-oxy C19 steroids as the predominant androgens in prostate cancer**

Therina du Toit, Amanda C. Swart

2018 – Molecular and Cellular Engineering

Keywords: 11keto-dihydrotestosterone (11KDHT) is readily inactivated *in vitro*; Dihydrotestosterone (DHT) is fully conjugated while 11KDHT remains unconjugated; C11-oxy C19 steroid levels are significantly higher than C19 steroid levels *in vivo*; Conjugated 11KDHT and 11ketotestosterone (11KT) levels are negligible in plasma; 11βHSD2 catalyses 11KDHT and 11KT biosynthesis in C4-2B and VCaP cells.

**Abstract**

Although the adrenal C19 steroids, androstenedione and testosterone, contribute to prostate cancer (PCa) progression the full complement of adrenal androgens, including the C11-oxy C19 steroids, 11β-hydroxyandrostenedione (11OHA4) and 11β-hydroxytestosterone (11OHT) and their androgenic metabolites, 11keto-testosterone (11KT) and 11keto-dihydrotestosterone (11KDHT) have, to date, not been considered. This study investigated the contribution of 11OHA4 and 11OHT to the pool of active androgens in the prostate. Steroid profiles were determined in LNCaP, C4-2B and VCaP cell models, in PCa tissue, and in plasma focussing on the inactivation, reactivation and glucuronidation of 11OHA4, 11OHT and their downstream products using ultra-performance convergence chromatography tandem mass spectrometry (UPC²-MS/MS). The C11-oxy C19 steroids were the predominant steroids with the production of 11KT and 11KDHT in prostate cell models identifying 11β-hydroxysteroid dehydrogenase type 2 activity. Active:inactive steroid ratios indicated efficient inactivation of dihydrotestosterone (DHT) and 11KDHT by 3α-hydroxysteroid dehydrogenases, while the reactivation of DHT by retinol-like dehydrogenases was greater than the reactivation of 11KDHT. In PCa tissue, inactive C11-oxy C19 steroids ranged from 27 to 30 ng/g, whereas inactive C19 steroids were below 1 ng/g. Steroid glucuronidation was impeded: in VCaP cells, the C11-oxy C19 steroids were unconjugated and the C19 steroids fully conjugated; in C4-2B cells, all steroids were unconjugated, except for DHT of which 50% was conjugated; in LNCaP cells only androsterone, 11KT and 11β-hydroxyandrosterone were unconjugated. In PCa patients’ plasma 11KDHT was present only in the unconjugated form, with 11KT also predominantly unconjugated (90–95%). Even though plasma and tissue sample numbers were limited, this study serves to demonstrate the abundance of C11-oxy C19 steroids, with notable differences in their metabolism, dictated by steroidogenic enzymes and hampered conjugation, affecting active androgen levels. Larger cohorts are required to analyse profiles in modulated metabolic pathways, in order to shed light on treatment outcomes. The C11-oxy C19 steroids are involved in PCa, with impeded glucuronidation in PCa ascribing a dominant role to these steroids in disease progression.
Improvements induced in lipid metabolism in the liver by D-47, a newly developed compound, were examined herein. WHHLMI rabbits, an animal model of hypercholesterolemia and coronary atherosclerosis, was fed D-47-supplemented chow for 5 weeks at a dose of 30 mg/kg. Lipid concentration were assayed using enzymatic methods. Plasma lipoproteins were fractionated with an ultracentrifuge. mRNA expression was analyzed with real-time PCR. Lipidome analyses of lipoproteins were performed using supercritical fluid chromatography mass spectrometry. In the D-47-treated group, serum lipid levels decreased by 23% for total cholesterol and by 40% for triglycerides. These reductions were mainly attributed to decreases in the VLDL fraction. Compared with the control, in the D-47 group, lipid contents in the liver were decreased by 22% in cholesterol and by 69% in triglycerides, and fat accumulation was decreased by 57% in pericardial fat and by 17% in mesenteric fat. In lipidome analyses of VLDL fraction, lysophosphatidylcholine, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylethanolamine plasmalogen, sphingomyelin, and ceramide were decreased by the D-47 treatment. mRNA expression in the liver was 51% lower for FAS and 24% lower for MTP, but 5.9- and 5.1-fold higher for CYP7A1 and CPT-1, respectively, in the D-47 group than in the control. mRNA expression was 72%, 64%, and 36% higher for LPL, CTP-1, and PPARγ, respectively, in mesenteric fat in the D-47 group.

D-47 is a potent lipid-lowering compound that uses a different mechanism of action from that of statins. It has potential as a compound in the treatment of steatohepatitis and metabolic syndrome.
11. Supercritical fluid chromatographic-tandem mass spectrometry method for monitoring dissipation of thiacloprid in greenhouse vegetables and soil under different application modes

Runan Li, Zenglong Chen, Fengshou Dong, Jun Xu, Xingang Liu, Xiaohu Wu, Xinglu Pan, Yan Tao, Yongquan Zheng

2018 – Journal of Chromatography B

Keywords: Thiacloprid, Greenhouse vegetables, Application modes, Degradation rates, SFC-MS/MS

Abstract

A rapid, sensitive and effective supercritical fluid chromatographic-tandem mass spectrometry (SFC-MS/MS) method was developed to analyze thiacloprid for the first time. The SFC-MS/MS conditions were optimized with the ultra-performance convergence chromatography (UPC²) BEH column (100 mm × 3.0 mm, 1.7 μm particle size) and thiacloprid was eluted at 1.22 min in gradient mode with CO₂/methanol as mobile phase. The 0.1% formic acid in methanol (v/v) was used as postcolumn compensation solution to improve sensitivity. The ABPR pressure, flow rate of mobile phase and flow rate of compensation pump were set at 1800 psi, 1.8 mL/min, and 0.1 mL/min, respectively. The average recoveries of thiacloprid in soil at four spiking levels (5, 10, 100, 1000 μg/kg) ranged between 78.8% and 107.1% with relative standard deviations (RSDs) lower than 12.2% and the limit of quantitation (LOQ) was 5 μg/kg. The proposed method can distinctly improve the analysis efficiency by 2–12 times and reduce the solvent consumption by 5%–95% compared with reported methods. It was applied to investigate the dissipation rates of thiacloprid in greenhouse vegetables and soil under different application modes. The half-lives of thiacloprid in cucumber and soil were 9.55–20.44 days and 3.74–9.14 days separately under different application modes, 10.60 days in tomato under foliar spraying. The residues in vegetables under root irrigation were all less than that under foliar spraying. The results could offer useful data for risk assessment of thiacloprid in agricultural production.

https://doi.org/10.1016/j.jchromb.2018.02.021

12. Analytical challenges to determine emerging persistent organic pollutants in aquatic ecosystems

María Lorenzo, Julián Campo, Yolanda Picóac

2018 – TrAC Trends in Analytical Chemistry

Keywords: Emerging persistent organic pollutants, Perfluoroalkyl substances, Novel brominated flame retardants, Organophosphorus flame retardants, Aquatic ecosystem, Sample treatment extraction, Analytical determination

Abstract

Emerging persistent organic pollutants (ePOPs) include polybrominated biphenyl (PBDEs) and perfluorooctane sulfonamide/perfluorooctane sulfonate (POSF/PFOS), which are newly listed in the Stockholm Convention. Other ePOPs, which have not been regulated, include organophosphate flame retardants (PFRs), novel brominated flame retardants (NBFRs) and other perfluoroalkyl substances (PFASs). Often ePOPs data related to occurrence, toxicity, impact or environmental behavior are insufficient or inadequate because of the lack of proper analytical methods to obtain them. Thus, a critical review of the analytical procedures proposed in the last
For determining ePOPs by chromatographic methods in the different compartments of the aquatic ecosystems is presented. The overall analytical procedure, from sampling to final determination, is emphasized presenting recent developments in the extraction, pre-concentration, and instrumental detection needed for the accurate quantification of ePOPs in environmental samples. Finally, this review examines the basic challenges we face in order to anticipate future directions and urgent needs of this field.

https://doi.org/10.1016/j.trac.2018.04.003

13. Milk lipidomics: What we know and what we don't
Zhiqian Liu, Simone Rochfort, Ben Cocks

Keywords: Milk, Lipidomics, Gas chromatography, Liquid chromatography, Mass spectrometry

Abstract
Bovine milk contains 3–5% of fat, of which the dominant portion (about 98%) is in the form of triacylglycerols, whereas polar lipids such as glycerophospholipids and sphingolipids are minor components (0.5–1%) of milk fat. Milk fat is thought to contain several thousand lipid species, making it the most complex material in nature in terms of lipid composition. Significant progress has been made in the past two decades in the identification and quantification of lipid species of milk thanks to the advance in analytical tools especially high-resolution mass spectrometers (MS). Currently, milk lipids are characterized mainly in two ways, i.e. global fatty acid composition profiling by gas chromatography and lipid molecular species identification and quantification by liquid chromatography tandem MS; the former provides information related to the physicochemical properties and nutritive quality of milk fat, whereas the latter provides the detailed chemical structure of lipid species. Until now, only about 400 lipid species have been described in bovine milk, with many low-abundance species remaining to be discovered. The merits and limitations of various separation techniques and different MS methodologies applied to lipid structural elucidation and quantification are critically reviewed and the challenging problems to be solved in milk lipidomic analysis highlighted.

https://doi.org/10.1016/j.plipres.2018.06.002

14. Ultra-performance supercritical fluid chromatography: A powerful tool for the enantioseparation of thermotropic fluorinated liquid crystals
Keywords: Supercritical fluid chromatography, Enantioseparation, Liquid crystalline materials, Chiral fluorinated liquid crystals, Amylose

Abstract
A fast and simple supercritical fluid chromatography method for the enantioseparation of twenty newly synthesized orthoconic antiferroelectric liquid crystals is reported for the first time. The effects of alkoxy spacer length and fluorine atom presence and position in the phenyl ring on chromatographic behavior were investigated. Baseline enantioseparation of all compounds was achieved using simple mobile phases consisting of carbon dioxide and alcohol as cosolvent on (3,5-dimethylphenylcarbamate) derivative of amylose as chiral stationary phase. The analysis times ranged from 2 to 4 and from 4 to 7 min for most samples when using methanol and propane-2-ol, respectively. The significant effect of cosolvent type on the enantioseparation of these compounds was assessed and partial complementarity of methanol and propane-2-ol was observed.

https://doi.org/10.1016/j.aca.2018.07.001

15. Dedicated comparisons of diverse polysaccharide- and zwitterionic Cinchona alkaloid-based chiral stationary phases probed with basic and ampholytic indole analogs in liquid and subcritical fluid chromatography mode
Attila Bajtai, Gyula Lajkó, István Szatmári, Ferenc Fülöp, Wolfgang Lindner, István Ilisz, Antal Pétera

Keywords: Enantiomer separation, Zwitterionic chiral stationary phases, Polysaccharide-based columns, Indole analogs, Comparative study

Abstract
Normal phase (NP) high-performance liquid and sub- and supercritical fluid chromatographic (both acronymed as SFC) methods have been developed for the enantiomer separation of three basic and three ampholytic structurally related C-3-substituted indole analogs on seven non-ionic (neutral) polysaccharide-based and two chemically entirely different zwitterionic Cinchona alkaloid- and sulfonic acid-based chiral stationary phases (CSPs). In a systematic fashion the effect of the composition of the mobile phase, the nature of the alcohol and amine additives on the retention characteristics and enantioselectivity of the ionizable analytes were investigated. On all studied polysaccharide-based CSPs the three ampholytes remained unretained in NP-LC mode, while they were nicely retained and resolved in SFC mode. These unexpected results underline a specific property of liquid CO₂ as bulk solvent in combination with alcohols as co-solvents and amine additives thus creating an environment around the chiral selector sites which support the retention of ampholytes. The zwitterionic CSPs worked equally well for the resolution of the basic and ampholytic analytes using a polar ionic mobile phase in both LC and SFC modes.

Results acquired by studying the effect of temperature were used to calculate the changes in standard enthalpy $\Delta(\Delta H^\circ)$, entropy $\Delta(\Delta S^\circ)$, and free energy $\Delta(\Delta G^\circ)$ applying van't Hoff plots. The
values of the thermodynamic parameters depended on the nature of selectors, the structure of analytes and the properties of the mobile phases. On polysaccharide-based CSPs and columns operated in NP-LC mode enthalpically-, whereas in SFC mode both enthalpically- and entropically-driven enantiomer separations were observed.

16. High-fast enantioselective determination of prothioconazole in different matrices by supercritical fluid chromatography and vibrational circular dichroism spectroscopic study
Ying Jiang, Jun Fan, Rujian He, Dong Guo, Tai Wang, Hui Zhang, Weiguang Zhang

Keywords: Prothioconazole, Supercritical fluid chromatography, Stereoselective quantification, Vibrational circular dichroism spectroscopy, Absolute configuration; Fast separation and quantification for prothioconazole enantiomers by SFC; Absolute configurations of two enantiomers were confirmed through VCD spectroscopy; Enantioselective determination of prothioconazole in two matrices; Analysis time through SFC was five-fold shorter than traditional HPLC.

Abstract
Herein, we developed a rapid supercritical fluid chromatography (SFC) method for chiral separation and enantioselective determination of prothioconazole in soil and tomatoes. The potential effects of chiral stationary phases, co-solvents, column temperature, and back pressure on enantioseparation of prothioconazole have been studied in detail. Two prothioconazole enantiomers were best separated on cellulose tris(3, 5-dimethylphenylcarbamate)-coated chiral stationary phase with CO$_2$-2-propanol (80:20, v/v) as the mobile phase, and the run time through SFC (about 4 min) was five-fold shorter than HPLC. Then, through comparing the experimental vibrational circular dichroism spectrum of the later-eluted component with the calculated pattern based on the (R)-configuration, it should be (R)-(−)-prothioconazole. Moreover, the modified QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction and cleanup procedures were applied in enantiomeric analysis of prothioconazole in two matrices. Good linearity ($R^2 \geq 0.9992$) and recoveries (91.84–101.66%, RSD ≤ 3.98%) for two enantiomers were achieved. This proposed method showed good accuracy and precision, and might be suitable for fast enantioselective determination and residual quantitative analysis of prothioconazole in food and environmental samples.
17. C11-oxy C19 and C11-oxy C21 steroids in neonates: UPC²-MS/MS quantification of plasma 11β-hydroxyandrostenedione, 11-ketotestosterone and 11-ketoprogesterone

Therina du Toit², Martijn J.J. Finken, Henrike M. Hamer, Annemieke C. Heijboer, Amanda C. Swart
2018 - Steroids

Keywords: Disorders of sexual development (DSD), Congenital adrenal hyperplasia (CAH), 11keto-dihydrotestosterone (11KDHT), Congenital hypogonadotropic hypogonadism (CHH), Newborn care 11β-hydroxysteroid dehydrogenase (11βHSD)

Abstract
The purpose of this study was to identify the C11-oxy C₁₉ and C11-oxy C₂₁ steroids in male and female neonate plasma. At birth, the most abundant C11-oxy steroids detected in neonatal plasma were 11β-hydroxyandrostenedione, ~13 nM, and 11-ketoprogesterone, ~23 nM. C11-oxy C₁₉ steroids were higher than C₁₉ steroids in neonatal plasma, 22.2 nM vs 5.4 nM. The inclusion of C11-oxy C₁₉ and C₂₁ steroid reference ranges in routine steroid analyses will assist the characterization of disorders associated with impaired steroidogenic enzyme expression and the identification of potential biomarkers.
18. Supercritical fluid chromatography coupled to mass spectrometry – A metabolomics perspective

Vladimir Shulaev, Giorgis Isaac

Keywords: Supercritical fluid chromatography, Mass spectrometry, Metabolomics, Metabolite profiling, SFC-MS, HPSFC-MS, UPC²-MS

Abstract

Metabolomics as a global analysis of a large number of cellular metabolites relies heavily on the new developments in separation science and technology. None of the existing analytical techniques can simultaneously separate and measure all the cellular metabolites due to complexity of cellular metabolome and, therefore, a combination of analytical techniques must be used. Currently NMR, GC–MS and LC-MS are most often used in metabolomics. Novel separation methods such as supercritical fluid chromatography (SFC), which can increase metabolome coverage while decreasing cost and analysis time, can provide alternative to other analytical techniques. As a result of major improvements in instrumentation and development of a new diverse column chemistries SFC-MS is increasingly used in a variety of biomedical applications and is becoming an attractive compliment to other major analytical platforms in metabolomics. Despite its potential and advantages, SFC-MS application in metabolomics is limited. Here we provide a brief overview of the latest developments of SFC-MS for metabolomics applications.

https://doi.org/10.1016/j.jchromb.2018.06.021

19. Methods in endogenous steroid profiling – A comparison of gas chromatography mass spectrometry (GC–MS) with supercritical fluid chromatography tandem mass spectrometry (SFC-MS/MS)

Julian Teubel, Bernhard Wüst, Carola G. Schipke, Oliver Peters, Maria Kristina Parr

Keywords: Successful separation of steroid isomers by SFC-ESI–MS/MS; Determination of 51 endogenous steroids and sulphates by SFC-ESI–MS/MS; Method development for determination of neurosteroids in cerebrospinal fluid; Method comparison of SFC-ESI–MS/MS and GC-EI-MS for steroid profiling; Intact steroid sulfate determination by SFC-MS/MS

Abstract

In various fields of endocrinology, the determination of steroid hormones synthesised by the human body plays an important role. Research on central neurosteroids has been intensified
within the last years, as they are discussed as biomarkers for various cognitive disorders. Their concentrations in cerebrospinal fluid (CSF) are considered to be regulated independently from peripheral fluids. For that reason, the challenging matrix CSF becomes a very interesting specimen for analysis. Concentrations are expected to be very low and available amount of CSF is limited. Thus, a comprehensive method for very sensitive quantification of a set of analytes as large as possible in one analytical aliquot is desired.

However, high structural similarities of the selected panel of 51 steroids and steroid sulfates, including numerous isomers, challenges achievement of chromatographic selectivity.

Since decades the analysis of endogenous steroids in various body fluids is mainly performed by gas chromatography (GC) coupled to (tandem) mass spectrometry (MS/(MS)). Due to the structure of the steroids of interest, derivatisation is performed to meet the analytical requirements for GC–MS/(MS). Most of the laboratories use a two-step derivatisation in multi-analyte assays that was already published in the 1980s. However, for some steroids this elaborate procedure yields multiple isomeric derivatives. Thus, some laboratories utilize (ultra) high performance liquid chromatography ((U)HPLC)–MS/MS as alternative but, even UHPLC is not able to separate some of the isomeric pairs. Supercritical fluid chromatography (SFC) as an orthogonal separation technique to GC and (U)HPLC may help to overcome these issues.

Within this project the two most promising methods for endogenous steroid profiling were investigated and compared: the “gold standard” GC–MS and the orthogonal separation technique SFC-MS/MS. Different derivatisation procedures for gas chromatographic detection were explored and the formation of multiple derivatives described and confirmed. Taken together, none of the investigated derivatisation procedures provided acceptable results for further method development to meet the requirements of this project. SFC with its unique selectivity was able to overcome these issues and to distinguish all selected steroids, including (pro-)gestagens, androgens, corticoids, estrogens, and steroid sulfates with appropriate selectivity. Valued especially in the separation of enantiomeric analytes, SFC has shown its potential as alternative to GC. The successful separation of 51 steroids and steroid sulfates on different columns is presented to demonstrate the potential of SFC in endogenous steroid profiling.

https://doi.org/10.1016/j.chroma.2018.04.035

20. Development of an analytical method for separation of phenolic acids by ultra-performance convergence chromatography (UPC²) using a column packed with a sub-2-μm particle

Hai Jiang, Liu Yang, Xudong Xing, Meiling Yan, Xinyue Guo, Bingyou Yang, Qiu-Hong Wang, Hai-Xue Kuanga

2018 – Journal of Pharmaceutical and Biomedical Analysis

Keywords: A method for the analysis of phenolic acids by ultra-performance convergence chromatography is firstly proposed; The developed method was subsequently applied to the determination of eight phenolic acids in 10 batch of Xanthii Fructus by ultra-performance convergence chromatography; The newly method is beneficial for quality control and
standardization of herbal drugs using UPC$^2$, providing an efficient, rapid and environmentally friendly scientific basis for future analysis of phenolic acids in TCM.

**Abstract**

Phenolic acids are important active components of certain Traditional Chinese Medicines (TCM) and have a wide range of biological effects. Separation and purification of phenolic acids remains challenging due to difficulties with quality control using existing chromatographic methods. The purpose of this study was to compare the effects of different chromatographic columns and conditions for the separation of phenolic acids. The BEH column was determined to be optimal, providing efficient separation in the shortest time (17.00 min) using gradient elution with carbon dioxide as the mobile phase, methanol/acetonitrile (70:30, v/v) with 1% TFA as the modifier, and a flow rate of 0.8 mL/min. Good peak shapes were obtained, and the peak asymmetry values were close to 1.00 for all phenolic acids. The resolution was more than 2.83 for all separated peaks. The developed method was subsequently applied to the determination of phenolic acids in Xanthii Fructus. These results are beneficial for quality control and standardization of herbal drugs using UPC$^2$, providing an efficient, rapid and environmentally friendly scientific basis for future analysis of phenolic acids.

21. A high-throughput UPC$^2$-MS/MS method for the separation and quantification of $C_{19}$ and $C_{21}$ steroids and their C11-oxy steroid metabolites in the classical, alternative, backdoor and 11OHA4 steroid pathways

Therina du Toit, Maria A. Stander, Amanda C. Swart

Journal of Chromatography B

**Keywords:**

UPC$^2$–MS/MS quantifies $C_{19}$ and $C_{21}$ steroids – including hydroxyl and keto steroids, UPC$^2$ separates 28 steroids in a serum matrix in a single 6 min chromatographic step, Sensitivity of the method: LOQs ranging from 0.01 to 20 ng/mL, Selectivity of the method separates stereoisomers and regioisomers.

**Abstract**

In the present study an ultra-performance convergence chromatography tandem mass spectrometry (UPC$^2$-MS/MS) analytical method was developed and validated for the determination of 17 $C_{19}$ and 14 $C_{21}$ steroids, including C11-oxy $C_{19}$ and C11-oxy $C_{21}$ steroids. The limit of detection and limit of quantification ranged from 0.01 to 10 ng/mL and from 0.01 to 20 ng/mL, respectively, and the method shows the recovery, matrix effect and process efficiency of steroids isolated from a serum matrix to be within acceptable limits. Good accuracy, repeatability and
reproducibility were also shown and the method provided excellent sensitivity and selectivity as stereoisomers and regioisomers were also resolved and quantified accurately. Clinical conditions such as congenital adrenal hyperplasia, polycystic ovary syndrome in females and disorders of sex development in neonates and in children, amongst others, are characterized by abnormal steroid levels. Steroid profiling is essential to accurately diagnose steroid levels in the above settings as well as in androgen excess or deficiency in adrenal-linked endocrine diseases. Our method, separating C\textsubscript{19} and C\textsubscript{21} steroids in a single chromatographic step, offers a reduced sample turnover rate in the clinical setting, while providing comprehensive steroid profiles of \textit{in vivo} steroids in the nmol/L range. This is, to our knowledge, the first method reported to simultaneously separate C\textsubscript{19} and C\textsubscript{21} steroids, together with their C11-hydroxy and C11-keto metabolites –one which may hold promise in the identification of new steroid markers in steroid-linked endocrine diseases, in addition to profiling steroid metabolism and abnormal enzyme activity in patients.

https://doi.org/10.1016/j.jchromb.2018.02.023

22. Exploring lipid markers of the quality of coix seeds with different geographical origins using supercritical fluid chromatography mass spectrometry and chemometrics
Jin-Jun Hou, Chun-Mei Cao, Yong-wei Xu, Shuai Yao, Lu-Ying Cai, Hua-Li Long, Qi-Rui Bi, Yuan-Yuan Zhen, Wan-Ying Wu, De-an Guo
2018 – Phytomedicine

Keywords: Traditional Chinese medicine, Lipids, Coix seeds, metabolites, CCD test, markers

Abstract

\textbf{Background:} Lipids, a group of primary metabolites, could be used as quality markers of Traditional Chinese medicine.

\textbf{Purpose:} The present study was designed to develop a research method to explore lipid markers of the quality of coix seeds with different geographical origins.

\textbf{Study Design:} The geographical origins of coix seeds were divided into three regions based on the latitude. A central composite design (CCD test) was used to optimize the chromatographic parameters of supercritical fluid chromatography to obtain optimal lipid profile of coix seed.

\textbf{Methods:} An untargeted method based on ultra-performance convergence chromatography - quadrupole/time-of-flight hybrid mass spectrometry (UPC\textsuperscript{2}-QTOF) was developed. Four chromatographic parameters were optimized using CCD test, and a fusion index established by
Derringer function was used to evaluate. The lipid profile of 27 batches of coix seeds were acquired and processed by Progenesis QI software, and the MS/MS spectrums were obtained to identify, simultaneously. The difference lipids were explored by orthogonal partial least squares discriminant analysis (OPLS-DA). The lipids that showed differences depending on their seeds’ geographical origin were selected as markers of the quality of coix seeds from the three regions. 

**Results:** A Torus 2-PIC (1.7 µm, 100 mm × 3.0 mm) was selected as the optimal column of the untargeted method which the run time was only 8 minutes. From the CCD test, the interaction of chromatographic parameters between column temperature and backpressure was founded which the optimal parameters were 55 °C and 2600 psi, respectively. Thirty-two peaks in the lipid profile of coix seed were tentatively identified, of which 20 were triglyceride, and 12 were diglyceride. Nine features that could potentially be used to distinguish the coix seeds by their geographical origin were identified, most of which were diglycerides, such as OP.

**Conclusions:** Our findings confirm that UPC²-QTOF combined with chemometrics could be used as an efficient method for exploring potential lipid markers of the quality of herbal medicine.

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### 23. Enantioselective behaviour of tetaconazole during strawberry wine-making process

**Na Liu, Xinglu Pan, Shuang Zhang, Mingshan Ji, Zhihong Zhang**

2018 – Chirality

**Keywords:** Chiral, Tetaconazole, wine making, strawberry

**Abstract**

The fate of tetaconazole enantiomers in strawberries during wine-making process was studied. The residues were determined by ultra-performance convergence chromatography tandem triple quadrupole mass spectrometry after each process steps. Results indicated that there was significant enantioselective dissipation of tetaconazole enantiomers during the fermentation process. And (−)-tetaconazole degraded faster than (+)-tetaconazole. The half-lives of (−)-tetaconazole and (+)-tetaconazole were 3.12, 3.76 days with washing procedure and 3.18, 4.05 days without washing procedure. The processing factors of strawberry wine samples after each step were generally less than 1. In particular, the processing factors of the fermentation process were the lowest. The results could help facilitate more accurate risk assessments of tetaconazole during wine-making process.

[https://doi.org/10.1002/chir.22845](https://doi.org/10.1002/chir.22845)

### 24. Improved Analytical Method for Dichlorobenzaldehyde Impurity Detection in Lozenges by Convergence Chromatography

**Nandan Kumar Dhir, Tulika Mishra, Varinder Kaur**

2018 - Asian Journal of Pharmaceutical Research and Health

**Keywords:** 2,4 - dichlorobenzaldehyde, Di Benzyl Alcohol, Gas Chromatography, Lozenges, Ultra Performance Convergence Chromatography

**Abstract**

Lozenges are the commonly used throat relieving pharmaceutical product that contains DBA and AMC as their active pharmaceutical ingredients. Reports have shown that during the longer storage conditions DBA converts to toxic 2, 4, dichlorobenzaldehyde (DCBZ). Present study reports the development of novel method for detection of DCBZ with UPC2 (Ultra performance convergence chromatography) instrument, that utilize the potential supercritical fluid
chromatography, which is sustainable, reduced cost and green technology reducing the use of organic solvent. In this simple and robust method to detect DCBZ, runtime reduced to 2 minutes as compared to 10 min by Gas Chromatography with higher precision. By utilizing conventional method of Gas Chromatography, 2, 4, dichlorobenzaldehyde is detectable but with very low response and interference with other ingredients use in Lozenges. Method has facilitated faster detection and more sensitivity for DCBZ detection. The improved method provides less flow rate during the process, making it highly cost-effective. Use of non-polar solvents, high to low gradient is some of the advantages of the improved method of present investigation when compared to traditional method. The improved process of present invention proves to be 27 times more cost-effective.

https://doi.org/10.18311/ajprhc/2018/18109

25. The utility of ultra-high performance supercritical fluid chromatography–tandem mass spectrometry (UHPSFC-MS/MS) for clinically relevant steroid analysis
2018 - Journal of Chromatography B

Keywords: Ultra-high performance supercritical fluid chromatography–tandem mass spectrometry, Ultra-high performance liquid chromatography–tandem mass spectrometry, Gas chromatography–mass spectrometry, Steroid analysis

Abstract
Liquid chromatography tandem mass spectrometry (LC-MS/MS) assays are considered the reference standard for serum steroid hormone analyses, while full urinary steroid profiles are only achievable by gas chromatography (GC–MS). Both LC-MS/MS and GC–MS have well documented strengths and limitations. Recently, commercial ultra-high performance supercritical fluid chromatography–tandem mass spectrometry (UHPSFC-MS/MS) systems have been developed. These systems combine the resolution of GC with the high-throughput capabilities of UHPLC. Uptake of this new technology into research and clinical labs has been slow, possibly due to the perceived increase in complexity. Here we therefore present fundamental principles of UHPSFC-MS/MS and the likely applications for this technology in the clinical research setting, while commenting on potential hurdles based on our experience to date.
26. A study on the onset of turbulent conditions with SFC mobile-phases

Keywords: SFC, Turbulent, Laminar, Pressure-drop, Extra-column, Tubing

Abstract
Following a recent publication [1], the topic of turbulent flow in SFC has generated both interest and questions. Liquid-like density, coupled with significantly low viscosity of CO2-based mobile-phases may result in high Reynolds number (Re) – higher than what represents laminar flow conditions, reaching the so-called turbulent regions. Although such turbulent flows can form only in the connecting tubings, thus not directly affecting the chromatographic process, it is important to know under many situations, whether the flow inside the tubing is laminar or turbulent. In this report a comprehensive guideline to identify the possibilities of turbulent flow conditions is provided through a series of charts. Flow properties depend on state conditions (composition, pressure and temperature) and also on the tubing material and geometry. Here guidelines to detect the onset of turbulent conditions is provided for cylindrical stainless-steel tubings of different internal diameters (i.d.) under a wide range of SFC mobile-phase conditions.

Highlights
• Turbulent flow strongly depends on set method conditions.
• Possibilities of turbulence higher in lower pressure and higher temperatures.
• Flow can be turbulent even with high percent of co-solvent.
• Charts, summarizing comprehensive effects of method conditions are presented.

https://doi.org/10.1016/j.chroma.2017.11.056

27. Characterization of five chemistries and three particle sizes of stationary phases used in supercritical fluid chromatography

Keywords: Linear solvation energy relationships (LSER), Solvation parameter model, Stationary phase characterization, Sub-2 microns particles, Supercritical fluid chromatography, Ultra-performance convergence chromatography

Abstract
Sub-2-microns particles employed as supporting phases are known to favor column efficiency. Recently a set of columns based on sub-2-microns particles for use with supercritical fluid mobile phases have been introduced by Waters. Five different stationary phase chemistries are available: BEH silica, BEH Ethyl-pyridine, XSelect CSH Fluorophenyl, HSS C18 SB and BEH Shield RP18. This paper describes the characterization of 15 stationary phases, the five different chemistries, and three particle sizes, 1.7 (or 1.8), 3.5 and 5 microns, with the same carbon dioxide–methanol
mobile phase and a set of more than a hundred compounds. The interactions established in the 15 different chromatographic systems used in supercritical fluid chromatography (SFC) are assessed with linear solvation energy relationships (LSERs). The results show the good complementarity of the five column chemistries, and their comparative location inside a classification map containing today around 70 different commercial phases. Among the five different chemistries, the HSS C18 SB phase displays a rather unusual behavior in regards of classical C18 phases, as it displays significant hydrogen–bonding interactions. Besides, it appears, as expected, that the BEH Ethyl–pyridine phase has weak interactions with basic compounds. The effect of particle size was studied because smaller particles induce increased inlet and internal pressure. For compressible fluids, this pressure change modifies the fluid density, i.e. the apparent void volume and the eluting strength. These changes could modify the retention and the selectivity of compounds in the case of method transfer, by using different particle sizes, from 5 down to 1.7 μm. A hierarchical cluster analysis shows that stationary phase clusters were based on the phase chemistry rather than on the particle size, meaning that method transfer from 5 to 1.7 microns can be achieved in the subcritical domain i.e. by using a weakly compressible fluid.

Highlights
• 5 different stationary phase chemistries were evaluated with 3 particle sizes.
• The systems were compared based on retention measured for over 100 analytes.
• Chemometric methods were used to assess the effects of changing particle size.
• Method transfer from 5 to 1.7 μm particles is possible with weakly compressible fluids.

https://doi.org/10.1016/j.chroma.2013.10.037

28. Supercritical fluid chromatography–tandem mass spectrometry-assisted methodology for rapid enantiomeric analysis of fenbuconazole and its chiral metabolites in fruits, vegetables, cereals, and soil

2017 – Food Chemistry

Keywords: Chiral, Enantioseparation, Fenbuconazole, Metabolites, SFC-MS/MS

Abstract
Here, we developed a rapid and robust supercritical fluid chromatography–tandem mass spectrometry (SFC-MS/MS) method for simultaneous detection of fenbuconazole and its chiral metabolites in fruit, vegetable, cereal, and soil. Baseline separation of six stereoisomers was achieved on an amylose tris-(3,5-dimethylphenylcarbamate)-coated chiral column in 4.0 min using a mobile phase composed of CO2/ethanol (flow rate of 1.8 mL/min). Ionisation efficiency and sensitivity was optimized with a post-column compensation solvent (0.1% formic acid/methanol). Target analytes were extracted with acetonitrile and purified using dispersive solid phase extraction sorbents. Six stereoisomers at three concentrations (5, 50, and 250 µg/kg) achieved satisfactory recoveries (76.3–104.6%) with RSDs ≤ 11.5% Excellent lineairties (R2 ≥ 0.9963) and the limits of quantification (LOQs, 0.13–3.31 µg/kg) were established for all six stereoisomers. Results show that the proposed method is suitable for routine detection of six stereoisomers of fenbuconazole and its chiral metabolites in food and environmental samples.

Highlights
• SFC-MS/MS was initially developed to analyze fenbuconazole and its metabolites.
• The total six stereoisomers were successfully baseline separated within 4.0 min.
• 0.4 mL/min flow rate of the compensation solvent showed higher MS signal response

https://doi.org/10.1016/j.foodchem.2017.08.038

29. Lipidomic analysis of biological samples: Comparison of liquid chromatography, supercritical fluid chromatography and direct infusion mass spectrometry methods

2017 – Journal of Chromatography A

Keywords: Lipidomics, Lipidomic analysis, UHPLC, UHPSFC, Direct infusion, Mass spectrometry

Abstract
Lipidomic analysis of biological samples in a clinical research represents challenging task for analytical methods given by the large number of samples and their extreme complexity. In this work, we compare direct infusion (DI) and chromatography – mass spectrometry (MS) lipidomic approaches represented by three analytical methods in terms of comprehensiveness, sample throughput, and validation results for the lipidomic analysis of biological samples represented by tumor tissue, surrounding normal tissue, plasma, and erythrocytes of kidney cancer patients.
Methods are compared in one laboratory using the identical analytical protocol to ensure comparable conditions. Ultrahigh-performance liquid chromatography/MS (UHPLC/MS) method in hydrophilic interaction liquid chromatography mode and DI-MS method are used for this comparison as the most widely used methods for the lipidomic analysis together with ultrahigh-performance supercritical fluid chromatography/MS (UHPSFC/MS) method showing promising results in metabolomics analyses. The nontargeted analysis of pooled samples is performed using all tested methods and 610 lipid species within 23 lipid classes are identified. DI method provides the most comprehensive results due to identification of some polar lipid classes, which are not identified by UHPLC and UHPSFC methods. On the other hand, UHPSFC method provides an excellent sensitivity for less polar lipid classes and the highest sample throughput within 10 min method time. The sample consumption of DI method is 125 times higher than for other methods, while only 40 μL of organic solvent is used for one sample analysis compared to 3.5 mL and 4.9 mL in case of UHPLC and UHPSFC methods, respectively. Methods are validated for the quantitative lipidomic analysis of plasma samples with one internal standard for each lipid class. Results show applicability of all tested methods for the lipidomic analysis of biological samples depending on the analysis requirements.

Highlights
- UHPLC, UHPSFC and direct infusion MS methods are compared for the lipidomic analysis of biological samples.
- Methods are tested in one laboratory using the identical analytical protocol and instrumentation.
- Comprehensiveness, sample throughput and validation results of individual methods are demonstrated.
- Tested methods are applicable for the lipidomic analysis of biological samples with particular limitations for each method.

https://doi.org/10.1016/j.chroma.2017.10.022

30. A rapid method for the separation of vitamin D and its metabolites by ultrahigh performance supercritical fluid chromatography–mass spectrometry

Keywords: Ultra-high performance supercritical fluid chromatography, Vitamin D2 and D3 metabolites, Orthogonal column screening, Method development, Plasma, Mass spectrometry

Abstract
In this study, a new supercritical fluid chromatography–mass spectrometry (SFC–MS) method has been developed for the separation of nine vitamin D metabolites within less than eight minutes. This is the first study of analysis of vitamin D and its metabolites carried out by SFC–MS. Six columns of orthogonal selectivity were examined, and the best separation was obtained by using a 1-aminoanthracene (1-AA) column. The number and the position of hydroxyl groups in the structure of the studied compounds as well as the number of unsaturated bonds determine the physiochemical properties and, thus the separation of vitamin D metabolites that is achieved on this column. All D2 and the D3 forms were baseline separated with resolution values > 1.5. The effects of pressure, temperature, flow rate and different gradient modes were studied. Electro spray ionization (ESI) and atmospheric pressure chemical ionization (APCI) were compared in positive mode, both by direct infusion and after SFC separation. The results showed that the sensitivity in APCI+ was higher than in ESI+ using direct infusion. In contrast, the sensitivity in APCI+ was 6-fold more sensitive than in ESI+ after SFC separation. The SFC–MS method was validated between 10 and 500 ng/mL for all analytes with coefficient of determination (R²) ≥ 0.999 for all calibration curves. The limits of detection (LOD) were found to range between 0.39 and 5.98 ng/mL for 24,25-dihydroxyvitamin D3 (24,25(OH)2D3) and 1-hydroxyvitamin D2 (1OH D2), respectively. To show its potential, the method was applied to human plasma samples from healthy individuals. Vitamin D3 (D3), 25-hydroxyvitamin D3 (25OHD3) and 24,25(OH)2D3 were determined in plasma samples and the concentrations were 6.6 ± 3.0 ng/mL, 23.8 ± 9.2 ng/mL and 5.4 ± 2.7 ng/mL, respectively.

Highlights
- Column screening on six orthogonal stationary phases for vitamin D analysis.
- Nine vitamin D metabolites (D2 and D3 forms) are baseline separated in 8 min.
- The effect of co-solvent, temperature, pressure and flow rate is studied.
- ESI+ is 6-fold more sensitive than APCI+ in SFC–MS.
31. Evaluation of the quantitative performances of supercritical fluid chromatography: From method development to validation

Keywords: Supercritical fluid chromatography (SFC), Ultra high performance supercritical fluid chromatography (UHPSFC), Ultra high performance liquid chromatography (UHPLC), Quantitative performances, Method validation, Total error approach

Abstract

Recently, the number of papers about SFC increased drastically but scientists did not truly focus their work on quantitative performances of this technique. In order to prove the potential of UHPSFC, the present work discussed about the different steps of the analytical life cycle of a method: from development to validation and application. Moreover, the UHPSFC quantitative performances were evaluated in comparison with UHPLC, which is the main technique used for quality control in the pharmaceutical industry and then could be considered as a reference. The methods were developed using Design Space strategy, leading to the optimization of robust method. In this context, when the Design Space optimization shows guarantee of quality, no more robustness study is required prior to the validation. Then, the methods were geometrically transferred in order to reduce the analysis time. The UHPSFC and UHPLC methods were validated based on the total error approach using accuracy profile. Even if UHPLC showed better precision and sensitivity, UHPSFC method is able to give accurate results in a dosing range larger than the 80–120% range required by the European Medicines Agency. Consequently, UHPSFC results are valid and could be used for the control of active substance in a finished pharmaceutical product. Finally, UHPSFC validated method was used to analyse real samples and gave similar results than the reference method (UHPLC).

Highlights

- UHPSFC method was successfully validated considering total error approach
- Quantitative performances of UHPSFC are compliant with the E.M.A. and ICH recommendations.
- UHPSFC can be used as a quantitative method for the quality control of medicines.
- Design Space strategy led to the optimization of a robust method as recommended by USP.

32. Analysis of hydroxylated polybrominated diphenyl ethers (OH-BDEs) by supercritical fluid chromatography/mass spectrometry

Keywords: Hydroxylated polybrominated diphenyl ethers (OH-BDEs), Supercritical fluid chromatography (SFC), Mass spectrometry (MS), Metabolism, Human serum

Abstract

Hydroxylated polybrominated diphenyl ethers (OH-BDEs), which have anthropogenic and natural origins, have exhibited neurotoxic and endocrine disrupting effects in humans and wildlife. Therefore, there is an increased interest in the analysis of these compounds in biological matrices in order to assess their potential toxicological risks. Analysis of OH-BDEs is conventionally completed using liquid chromatography/mass spectrometry (LC/MS), or by gas chromatography/mass spectrometry (GC/MS) after derivatization. Issues with resolution in separating congeners have limited the analysis of OH-BDEs via LC/MS, with published methods only able to include 13 congeners in the analysis. On the other hand, while GC/MS analysis can analyze more OH-BDE congeners, derivatization of OH-BDEs to convert them to GC amenable compounds adds to sample preparation time and limits the column lifetime due to trace residues of highly reactive derivatization agents entering the column. Herein we report the development of a supercritical fluid chromatography/mass spectrometry (SFC/MS) method for the analysis of 22 OH-BDE congeners. Instrument limits of detection for the developed method ranged from 2 to 106 fg injected on column, which is lower than previously optimized LC/MS and GC/MS methods. The developed SFC/MS method was successfully applied towards the analysis of in vitro metabolism samples and human serum samples to demonstrate its applicability with different biological matrices.
Highlights
- The development of an SFC/MS/MS method for the analysis of OH-BDEs.
- Calculated iLODs ranged from 2 to 106 fg injected on column.
- Successfully applied towards in vitro metabolism and human serum samples.

https://doi.org/10.1016/j.talanta.2016.08.013

33. Coupling state-of-the-art supercritical fluid chromatography and mass spectrometry: From hyphenation interface optimization to high-sensitivity analysis of pharmaceutical compounds

Keywords: SFC–MS, UHPSFC–MS, Interfacing approach, Detection sensitivity, Pharmaceutical application

Abstract

The recent market release of a new generation of supercritical fluid chromatography (SFC) instruments compatible with state-of-the-art columns packed with sub-2 μm particles (UHPSFC) has contributed to the reemergence of interest in this technology at the analytical scale. However, to ensure performance competitiveness of this technique with modern analytical standards, a robust hyphenation of UHPSFC to mass spectrometry (MS) is mandatory. UHPSFC–MS hyphenation interface should be able to manage the compressibility of the SFC mobile phase and to preserve as much as possible the chromatographic separation integrity. Although several interfaces can be envisioned, each will have noticeable effects on chromatographic fidelity, flexibility and user-friendliness. In the present study, various interface configurations were evaluated in terms of their impact on chromatographic efficiency and MS detection sensitivity. An interface including a splitter and a make-up solvent inlet was found to be the best compromise and exhibited good detection sensitivity while maintaining more than 75% of the chromatographic efficiency. This interface was also the most versatile in terms of applicable analytical conditions. In addition, an accurate model of the fluidics behavior of this interface was created for a better understanding of the influence of chromatographic settings on its mode of operation. In the second part, the most influential experimental factors affecting MS detection sensitivity were identified and optimized using a design-of-experiment approach. The application of low capillary voltage and high desolvation temperature and drying gas flow rate were required for optimal ESI ionization and nebulization processes. The detection sensitivity achieved using the maximized UHPSFC–ESI-MS/MS conditions for a mixture of basic pharmaceutical compounds showed 4- to 10-fold improvements in peak intensity compared to the best performance achieved by UHPLC–ESI-MS/MS with the same MS detector.

Highlights
- Several UHPSFC–MS hyphenation interfaces were critically evaluated.
- The interface including a splitter and a make-up solvent inlet was found to be the most versatile.
- High desolvation temperature and drying gas flow rate were mandatory for optimal ESI process.
- UHPSFC–ESI-MS/MS was, on average, 10 times more sensitive than UHPLC–ESI-MS/MS.
UHPSFC–ESI-MS/MS appears to be a competitive analytical approach.
https://doi.org/10.1016/j.chroma.2014.03.006

34. Comparison of ultra-high performance supercritical fluid chromatography and ultra-high performance liquid chromatography for the separation of spirostanol saponins

2017 - Journal of Pharmaceutical and Biomedical Analysis

Keywords: Spirostanol saponins, Ultra-high performance supercritical fluid chromatography, Ultra-high performance liquid chromatography, Chromatographic behavior, Natural products

Abstract
Spirostanol saponins are important active components of some herb medicines, and their isolation and purification are crucial for the research and development of traditional Chinese medicines. We aimed to compare the separation of spirostanol saponins by ultra-high performance supercritical fluid chromatography (UHPSFC) and ultra-high performance liquid chromatography (UHPLC). Four groups of spirostanol saponins were separated respectively by UHPSFC and UHPLC. After optimization, UHPSFC was performed with a HSS C18 SB column or a Diol column and with methanol as the co-solvent. A BEH C18 column and mobile phase containing water (with 0.1% formic acid) and acetonitrile were used in UHPLC. We found that UHPSFC could be performed automatically and quickly. It is effective in separating the spirostanol saponins which share the same aglycone and vary in sugar chains, and is very sensitive to the number and the position of hydroxyl groups in aglycones. However, the resolution of spirostanol saponins with different aglycones and the same sugar moiety by UHPSFC was not ideal and could be resolved by UHPLC instead. UHPLC is good at differentiating the variation in aglycones, and is influenced by double bonds in aglycones. Therefore, UHPLC and UHPSFC are complementary in separating spirostanol saponins. Considering the naturally produced spirostanol saponins in herb medicines are different both in aglycones and in sugar chains, a better separation can be achieved by combination of UHPLC and UHPSFC. UHPSFC is a powerful technique for improving the resolution when UHPLC cannot resolve a mixture of spirostanol saponins and vice versa.

Highlights

- UHPSFC is a good automatic chromatographic technique and has little in common with normal phase HPLC.
- UHPSFC separates spirostanol saponins varying only in sugar chains or in hydroxyls of aglycones well.
- UHPLC with C18 column separates spirostanol saponins varying only in aglycones well, especially with different double bonds.
- UHPSFC and UHPLC are complementary and could be applied together in separating spirostanol saponins.

https://doi.org/10.1016/j.jpba.2015.12.002

35. Analysis of ultra-short chain perfluoroalkyl substances in Swedish environmental waters
The purpose of this study was to investigate the environmental occurrence of ultra-short chain perfluoroalkyl substances (PFASs) in Swedish water samples. So far established protocols have focused on measuring PFASs with a carbon chain length of four or more carbons. In this study, perfluoroalkyl sulfonates of chain lengths of two, perfluoroethane sulfonate (PFEtS), and three, perfluoropropane sulfonate (PFPrS), carbons have been measured using a newly established instrumental method employing supercritical fluid separation (SFC) coupled to tandem mass spectrometry detection. A total of 26 samples were analysed, including ground water, surface water, rain water and snow. The sample locations included military and civilian airports, a former hard chromium plating facility, the vicinity of a hazardous waste management facility and background areas (lake surface water, rain and snow). Results show that both PFPrS and PFEtS could be detected in environmental samples using SFC separation coupled to triple quadrupole detection. Out of the 26 samples analysed, the ultra-shortchain PFPrS could be detected and quantified in 22 samples. The concentrations for PFPrS in all the samples ranged between 0.93 ng/l to 39 000 ng/l. The ultra-short-chain PFEtS could be quantified in all of the 26 samples, with a concentration range between 0.07 and 5 700 ng/l. The highest concentrations represents highly contaminated ground water samples collected from a military airport. In the samples, PFPrS had a relative contribution to total PFAS concentration of 6 and 10 %, indicating the importance of measuring these compounds in environmental samples.

A rapid and sensitive supercritical fluid chromatography/tandem mass spectrometry method for detection of ezetimibe in dog plasma and its application in pharmacokinetic studies

Keywords: ezetimibe, SFC-MS/MS, beagle dog, pharmacokinetics

Abstract

The aim of this study is to develop and validate a rapid, high-selective and sensitive supercritical fluid chromatography/tandem mass spectrometry (SFC-MS/MS) with a multiple reactions monitoring (MRM) mode method for the detection of ezetimibe in dog plasma. Several conditions were optimized systematically as follows: lipid-lipid extraction (LLE) performances were used to extract analytes from dog plasma; an ACQUITY HSS C18 SB (1.8 μm, 3.0 × 100 mm) column was employed to separate the target compounds; the triple-quadrupole mass spectrometry equipped with electrospray ionization (ESI) source was applied to detect ezetimibe. The method, which required a relatively small volume of plasma (100 μL), was obtained at concentration ranging from 1.0 to 100 ng/mL(r² > 0.99). The lower limit of quantification (LLOQ)for ezetimibe was found to be as low as 1.0 ng/mL. In addition, the validations of the methodology including sensitivity, recovery, matrix effect, intra- and inter-day precision, accuracy and stability were all within acceptable limits. The Cmax, AUC0–inf and Tmax values obtained in our study were 52.2 ± 6.3, 820.6 ± 4.3 and 1.25 ± 0.35 for reference formulation; 61.8 ± 12.6, 924.2 ± 4.7 and 2.00 ± 0 for test formulation. In conclusion, the method developed in this study can be successfully applied to pharmacokinetic studies after oral administration of ezetimibe in dogs.

Highlights

- We determined ezetimibe in dog plasma by a rapid, high-selective and sensitive SFC–MS/MS.
- Ezetimibe-glucuronide should be hydrolyzed to the original drug by the β-Glucuronidase.
- The total free ezetimibe will be detected by MS after liquid-liquid extraction.

https://doi.org/10.1016/j.jchromb.2017.10.053

Lipidomic analysis of biological samples: Comparison of liquid chromatography, supercritical fluid chromatography and direct infusion mass spectrometry methods

Keywords: Lipidomics, Lipidomic analysis, UHPLC, UHPSFC, Direct infusion, Mass spectrometry

Abstract

Lipidomic analysis of biological samples in a clinical research represents challenging task for analytical methods given by the large number of samples and their extreme complexity. In this

https://doi.org/10.1016/j.chroma.2017.03.029
work, we compare direct infusion (DI) and chromatography – mass spectrometry (MS) lipidomic approaches represented by three analytical methods in terms of comprehensiveness, sample throughput, and validation results for the lipidomic analysis of biological samples represented by tumor tissue, surrounding normal tissue, plasma, and erythrocytes of kidney cancer patients. Methods are compared in one laboratory using the identical analytical protocol to ensure comparable conditions. Ultrahigh-performance liquid chromatography/MS (UHPLC/MS) method in hydrophilic interaction liquid chromatography mode and DI-MS method are used for this comparison as the most widely used methods for the lipidomic analysis together with ultrahigh-performance supercritical fluid chromatography/MS (UHPSFC/MS) method showing promising results in metabolomics analyses. The nontargeted analysis of pooled samples is performed using all tested methods and 610 lipid species within 23 lipid classes are identified. DI method provides the most comprehensive results due to identification of some polar lipid classes, which are not identified by UHPLC and UHPSFC methods. On the other hand, UHPSFC method provides an excellent sensitivity for less polar lipid classes and the highest sample throughput within 10 min method time. The sample consumption of DI method is 125 times higher than for other methods, while only 40 μL of organic solvent is used for one sample analysis compared to 3.5 mL and 4.9 mL in case of UHPLC and UHPSFC methods, respectively. Methods are validated for the quantitative lipidomic analysis of plasma samples with one internal standard for each lipid class. Results show applicability of all tested methods for the lipidomic analysis of biological samples depending on the analysis requirements.

Highlights
- UHPLC, UHPSFC and direct infusion MS methods are compared for the lipidomic analysis of biological samples.
- Methods are tested in one laboratory using the identical analytical protocol and instrumentation.
- Comprehensiveness, sample throughput and validation results of individual methods are demonstrated.
- Tested methods are applicable for the lipidomic analysis of biological samples with particular limitations for each method.

https://doi.org/10.1016/j.chroma.2017.10.022

38. Ultra-high-performance supercritical fluid chromatography with quadrupole-time-of-flight mass spectrometry (UHPSFC/QTOF-MS) for analysis of lignin-derived monomeric compounds in processed lignin samples

Keywords: Column selectivity, Design of experiment, Ionisation efficiency, Lignin, Supercritical fluid chromatography

Abstract
The conversion of lignin to potentially high-value low molecular weight compounds often results in complex mixtures of monomeric and oligomeric compounds. In this study, a method for the quantitative and qualitative analysis of 40 lignin-derived compounds using ultra-high-performance supercritical fluid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UHPSFC/QTOF-MS) has been developed. Seven different columns were explored for maximum selectivity. Makeup solvent composition and ion source settings were optimised using a D-optimal design of experiment (DoE). Differently processed lignin samples were analysed and used for the method validation. The new UHPSFC/QTOF-MS method showed good separation of the 40 compounds within only 6-min retention time, and out of these, 36 showed high ionisation efficiency in negative electrospray ionisation mode.

https://doi.org/10.1007/s00216-017-0663-5
A rapid and selective method for the quantitative and qualitative analysis of 40 lignin-derived compounds using ultra-high-performance supercritical fluid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UHPSFC/QTOF-MS)

39. Method optimization for drug impurity profiling in supercritical fluid chromatography: Application to a pharmaceutical mixture

Keywords: Supercritical fluid chromatography, Separation optimization, Experimental design, Chromatographic response modeling, Chromatogram simulation

Abstract
A supercritical chromatographic method for the separation of a drug and its impurities has been developed and optimized applying an experimental design approach and chromatogram simulations. Stationary phase screening was followed by optimization of the modifier and injection solvent composition. A design-of-experiment (DoE) approach was then used to optimize column temperature, back-pressure and the gradient slope simultaneously. Regression models for the retention times and peak widths of all mixture components were built. The factor levels for different grid points were then used to predict the retention times and peak widths of the mixture components using the regression models and the best separation for the worst separated peak pair in the experimental domain was identified.
A plot of the minimal resolutions was used to help identifying the factor levels leading to the highest resolution between consecutive peaks. The effects of the DoE factors were visualized in a way that is familiar to the analytical chemist, i.e. by simulating the resulting chromatogram. The mixture of an active ingredient and seven impurities was separated in less than eight minutes. The approach discussed in this paper demonstrates how SFC methods can be developed and optimized efficiently using simple concepts and tools.

Highlights
- Effect of different modifier and injection solvent compositions on retention and peak shape.
- Optimization of temperature, back-pressure and gradient time with a response surface design approach.
- Modelling retention as a function of temperature, back-pressure and gradient time.
- Chromatogram simulation of the predicted optimal separation.

https://doi.org/10.1016/j.chroma.2017.10.036

40. Separation of achiral analytes using supercritical fluid chromatography with chiral stationary phases

Keywords: Achiral separation, Chiral stationary phase, Chiral supercritical fluid chromatography, Closely-related species, Complex mixture, Method development, Supercritical fluid chromatography, Ultra high performance liquid chromatography

Abstract
In recent years, chiral supercritical fluid chromatography (SFC) has emerged as the preferred technique for analytical, semi-preparative and preparative separation of enantiomers in the pharmaceutical industry, due to advantages in speed, high column efficiency and significantly lower mobile-phase consumption than conventional liquid chromatography (LC) techniques. We illustrate the benefits of SFC using chiral stationary phases (CSPs) in method development for separating multicomponent mixtures of closely-related achiral analytes, including hydroxylation isomers, halogen-containing molecules, drug metabolites and analogs, methylation and demethylation species, constitutional isomers, and diastereomers. We present several case studies to illustrate the advantage of using SFC with CSPs for achiral separations, where conventional achiral LC and achiral SFC methods fail or deliver sub-optimal chromatographic performance.

Highlights
- Solving multicomponent achiral separation challenges via SFC with chiral stationary phases (CSPs).
- Challenging mixtures of achiral drug metabolites and analogs can often be resolved by CSPs in SFC mode.
- Achiral UHPLC and SFC vs chiral SFC for separation of closely related achiral analytes.
- Separation of complex non-enantiomeric mixtures often require alternative approaches such as chiral SFC.

https://doi.org/10.1016/j.trac.2015.01.004

41. Simultaneous determination of topiramate, carbamazepine, oxcarbazepine and its major metabolite in human plasma by SFC-ESI-MS/MS with polarity switching: Application to therapeutic drug monitoring

2017 - Arabian Journal of Chemistry

Keywords: Antiepileptic drugs, Antiepileptic drugs, SFC-ESI-MS/MS, Human plasma, Therapeutic drug monitoring, UPC2

Abstract
Antiepileptic drugs are the first choice for epilepsy treatment. Monitoring antiepileptic drugs is important to minimize their adverse side effects by choosing the optimum drug dosage. An accurate and high throughput supercritical fluid chromatography-tandem mass spectrometry method has been developed for the simultaneous quantification of several antiepileptic drugs in human plasma. Plasma samples were extracted with ethyl acetate and the upper organic layer was directly injected into the supercritical fluid chromatography/mass spectrometry (SFC-MS/MS) system without further nitrogen evaporation and subsequent reconstitution. The analytes were eluted on a UPC2™ BEH, 2-EP column (100 × 3 mm, 1.7 μm) at a flow rate of 1.0 mL/min and multi-reaction monitoring (MRM) was performed for determination of the analytes and internal standard (IS) in polarity switching mode. Calibration curves were linear over the concentration ranges of 0.08–40, 0.01–15, 0.01–8 and 0.5–50 μg/mL with lower limit of quantifications of 0.08, 0.01, 0.01 and 0.50 μg/mL for topiramate, carbamazepine, oxcarbazepine and monohydroxycarbamazepine, respectively. This sensitive, accurate, novel method will be very useful for monitoring the above antiepileptic drugs and for pharmacokinetic studies.

https://doi.org/10.1016/j.arabjc.2016.09.016

42. Development and optimization of ultra-high performance supercritical fluid chromatography mass spectrometry method for high-throughput determination of tocopherols and tocotrienols in human serum

2017 – Analytica Chimica Acta

Keywords: Ultra-high performance supercritical fluid chromatography, Mass spectrometry, Liquid liquid extraction, Tocopherols, Tocotrienols, Human serum

Abstract
The goal of this study was to develop an effective supercritical fluid chromatography method using single quadrupole MS for analysis of all isomeric forms of vitamin E. Finally, two fast and effective methods, the high resolution one and the high speed one, for the determination of 8 vitamin E isomers in human serum were developed. Rapid high-throughput liquid-liquid extraction was selected as a sample preparation step. Sample pretreatment of 100 μL human serum was consisted of protein precipitation with 200 μL ethanol and liquid-liquid extraction by 400 μL hexane/dichloromethane (80/20, v/v). The separation was
performed on BEH 2-EP (3.0 × 100 mm, 1.7 μm) stationary phase, using isocratic elution with carbon dioxide and 10 mM ammonium formate in methanol in the ratio 98:2 for high resolution method with run time 4.5 min and in the ratio 95:5 for high speed method, where the run time was 2.5 min. The method development included optimization of key parameters: the choice of the suitable stationary phase and the composition of mobile phase, where an influence of various modifiers, their ratio and additives were tested, and optimization of fine tuning parameters including BPR pressure, flow-rate and column temperature. Quantification of all isomeric forms was performed using SIM (single ion monitoring) experiments in ESI positive ion mode. Both high speed and high resolution chromatographic methods were validated in terms of precision, accuracy, range, linearity, LOD, LOQ and matrix effects using the same LLE procedure. The high resolution method provided more sensitive results (LOD: 0.017–0.083 μg mL⁻¹) and better linearity (r² > 0.9930) than the high speed one (LOD: 0.083–0.25 μg mL⁻¹, r² > 0.9877) at the cost of double time of analysis.

Highlights
- Two fast, selective and sensitive UHPSFC-MS methods were developed and validated.
- Eight isomeric forms of vitamin E were fully separated in less than 4.5 min.
- The influence of individual SFC parameters was evaluated in detail.
- High-throughput LLE was used for sample preparation with sufficient selectivity and sensitivity.

https://doi.org/10.1016/j.aca.2016.06.008

43. Characterization of carotenoids in Rhodothermus marinus

Keywords: ACQUITY UPC2, Xevo G2-QToF,

Abstract
Rhodothermus marinus, a marine aerobic thermophile, was first isolated from an intertidal hot spring in Iceland. In recent years, the R. marinus strain PRI 493 has been genetically modified, which opens up possibilities for targeted metabolic engineering of the species, such as of the carotenoid biosynthetic pathway. In this study, the carotenoids of the R. marinus type-strain DSM 4252ᵀ, strain DSM 4253, and strain PRI 493 were characterized. Bioreactor cultivations were used for pressurized liquid extraction and analyzed by ultra-high performance supercritical fluid chromatography with diode array and quadrupole time-of-flight mass spectrometry detection (UHPSFC-DAD-QTOF/MS). Salinixanthin, a carotenoid originally found in Salinibacter ruber and previously detected in strain DSM 4253, was identified in all three R. marinus strains, both in the hydroxylated and nonhydroxylated form. Furthermore, an additional and structurally distinct carotenoid was detected in the three strains. MS/MS fragmentation implied that the mass difference between salinixanthin and the novel carotenoid structure corresponded to the absence of a 4-keto group on the ß-ionone ring. The study confirmed the lack of carotenoids for the strain SB-71 (ΔtrpBΔpurAcrtBI'::trpB) in which genes encoding two enzymes of the proposed pathway are partially deleted. Moreover, antioxidant capacity was detected in extracts of all the examined R. marinus strains and found to be 2–4 times lower for the knock-out strain SB-71. A gene cluster with 11 genes in two operons in the R. marinus DSM 4252ᵀ genome was identified and analyzed, in which several genes were matched with carotenoid biosynthetic pathway genes in other organisms.
44. The differences in matrix effect between supercritical fluid chromatography and reversed phase liquid chromatography coupled to ESI/MS

Keywords: Matrix effects, Supercritical fluid chromatography, Electrospray ionization, Liquid chromatography, Ion enhancement, Ion suppression

Abstract
For many sample matrices, matrix effects are a troublesome phenomenon using the electrospray ionization source. The increasing use of supercritical fluid chromatography with CO₂ in combination with the electrospray ionization source for MS detection is therefore raising questions: is the matrix effect behaving differently using SFC in comparison with reversed phase LC? This was investigated using urine, plasma, influent- and effluent-wastewater as sample matrices. The matrix effect was evaluated using the post-extraction addition method and through post-column infusions. Matrix effect profiles generated from the post-column infusions in combination with time of flight-MS detection provided the most valuable information for the study. The combination of both qualitative and semi-quantitative information with the ability to use HRMS-data for identifying interfering compounds from the same experiment was very useful, and has to the authors’ knowledge not been used this way before. The results showed that both LC and SFC are affected by matrix effects, however differently depending on sample matrix. Generally, both suppressions and enhancements were seen, with a higher amount of enhancements for LC, while 65% of all compounds and all sample matrices were enhanced, compared to only 7% for SFC. Several interferences were tentatively identified, with phospholipids, creatinine, and metal ion clusters as examples of important interferences, with different impact depending on chromatographic technique. SFC needs a different strategy for limiting matrix interferences, owing to its almost reverse retention order compared to RPLC.

Highlights;
- Matrix effects were compared using screening methods with SFC/ESI-MS and RPLC/ESI-MS.
- Blood plasma, horse urine and influent/effluent wastewater were investigated.
- Through the use of post-column infusions, matrix effect profiles were generated.
- Quantitative and qualitative information was compared, interferences tentatively identified.
- Ion suppressions were generally more common for SFC, and enhancements for LC.

https://doi.org/10.1016/j.aca.2017.10.014

45. Quantitative Profiling Of Endogenous Fat-Soluble Vitamins And Carotenoids In Human Plasma Using An Improved UHPSFC-ESI-MS Interface

Keywords: UPC2, Mass Spec interface

Abstract

Page 55 of 195
Analytical solutions enabling the quantification of circulating levels of liposoluble micronutrients such as vitamins and carotenoids are currently limited to either single or a reduced panel of analytes. The requirement to use multiple approaches hampers the investigation of the biological variability on a large number of samples in a time and cost efficient manner. With the goal to develop high-throughput and robust quantitative methods for the profiling of micronutrients in human plasma, we introduce a novel, validated workflow for the determination of 14 fat-soluble vitamins and carotenoids in a single run. Automated supported liquid extraction was optimized and implemented to simultaneously parallelize 48 samples in 1 h, and the analytes were measured using ultrahigh-performance supercritical fluid chromatography coupled to tandem mass spectrometry in less than 8 min. An improved mass spectrometry interface hardware was built up to minimize the post-decompression volume and to allow better control of the chromatographic effluent density on its route toward and into the ion source. In addition, a specific make-up solvent condition was developed to ensure both analytes and matrix constituents solubility after mobile phase decompression. The optimized interface resulted in improved spray plume stability and conserved matrix compounds solubility leading to enhanced hyphenation robustness while ensuring both suitable analytical repeatability and improved the detection sensitivity. The overall developed methodology gives recoveries within 85–115%, as well as within and between-day coefficient of variation of 2 and 14%, respectively.

http://pubs.acs.org/doi/abs/10.1021/acs.analchem.7b01476

46. Maternal And Female Fetal Testosterone Levels Are Associated With Maternal Age And Gestational Weight Gain

Keywords: UPC2, Testosterone, Cortisol

Abstract

OBJECTIVE: Prenatal androgen exposure has been suggested to play a role in polycystic ovary syndrome. Given the limited information on what maternal characteristics influence maternal testosterone levels, and the even less explored routes by which female fetus androgen exposure would occur, the aim of this study was to investigate the impact of maternal age, BMI, weight gain, depressed mood and aromatase SNPs on testosterone levels in maternal serum and amniotic fluid of female fetuses.

METHODS: Blood samples from pregnant women (n = 216) obtained in gestational weeks 35-39, and pre-labor amniotic fluid samples from female fetuses (n = 56), taken at planned Caesarean section or in conjunction with amniotomy for induction of labor, were analyzed. Maternal serum testosterone and amniotic fluid testosterone and cortisol were measured by tandem mass spectrometry.

RESULTS: Multiparity (β = -0.28, P < 0.001), self-rated depression (β = 0.26, P < 0.001) and weight gain (β = 0.18, P < 0.05) were independent explanatory factors for the maternal total testosterone levels. Maternal age (β = -0.34, P < 0.001), weight gain (β = 0.19, P < 0.05) and amniotic fluid cortisol levels (β = 0.44, P < 0.001) were independent explanatory factors of amniotic fluid testosterone in female fetuses, explaining 64.3% of the variability in amniotic fluid testosterone.

WIDER IMPLICATIONS OF THE FINDINGS: Young maternal age and excessive maternal weight gain may increase the prenatal androgen exposure of female fetuses. Further studies are needed to explore this finding.


47. QTL Mapping Of Stress Related Gene Expression In A Cross Between Domesticated Chickens And Ancestral Red Junglefowl.

Keywords: Hormonal analysis, UPC2

Abstract

Domestication of animals is associated with numerous alterations in physiology, morphology, and behavior. Lower reactivity of the hypothalamic-pituitary-adrenal (HPA) axis and reduced fearfulness is seen in most studied domesticates, including chickens. Previously we have shown that the physiological stress response as well as expression levels of hundreds of genes in the hypothalamus and adrenal glands are different between domesticated White Leghorn and the progenitor of modern chickens, the Red Junglefowl. To map genetic loci associated with the
transcription levels of genes involved in the physiological stress response, we conducted an eQTL analysis in the F12 generation of an inter-cross between White Leghorn and Red Junglefowl. We selected genes for further studies based on their known function in the regulation of the HPA axis or sympathoadrenal (SA) system, and measured their expression levels in the hypothalamus and the adrenal glands after a brief stress exposure (physical restraint). The expression values were treated as quantitative traits for the eQTL mapping. The plasma levels of corticosterone were also assessed. We analyzed the correlation between gene expression and corticosterone levels and mapped eQTLs and their potential effects on corticosterone levels. The effects on gene transcription of a previously found QTL for corticosterone response were also investigated. The expression levels of the glucocorticoid receptor (GR) in the hypothalamus and several genes in the adrenal glands were correlated with the post-stress levels of corticosterone in plasma. We found several cis- and trans-acting eQTL for stress-related genes in both hypothalamus and adrenal. In the hypothalamus, one eQTL for c-FOS and one QTL for expression of GR were found. In the adrenal tissue, we identified eQTL for the genes NR0B1, RGS4, DBH, MAOA, GRIN1, GABRB2, GABRB3, and HSF1. None of the found eQTL were significant predictors of corticosterone levels. The previously found QTL for GR expression in hypothalamus combined with the negative correlation between GR expression and corticosterone response suggests GR as a candidate for further functional studies regarding modification of stress response during chicken domestication.


Keywords: steroids, cortisol, cortisone, UPC2

Abstract

Maternal serum cortisol has been suggested to be influenced by psychiatric morbidity, and may also influence fetal growth. However, several studies found equal cortisol levels in depressed and healthy pregnant women. Placental 11-β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) shields the fetus from maternal cortisol by conversion to cortisone, a function that may be compromised by maternal stress. We aimed to compare the serum ratio of cortisone to cortisol, in women with and without psychiatric morbidity during pregnancy. A secondary aim was to investigate whether fetal growth, approximated by infant birth weight, was associated with the cortisone to cortisol ratio. We performed tandem mass spectrometry analysis of serum cortisol and cortisone in late pregnancy in 94 women with antenatal psychiatric morbidity and 122 controls (cohort 1). We also compared the placental gene expression of HSD11B1 and 2 in another group of 69 women with psychiatric morbidity and 47 controls (cohort 2). There were no group differences in cortisone to cortisol ratio, absolute levels of cortisone and cortisol (cohort 1), or expression of HSD11B1 or 2 (cohort 2). However, cortisone to cortisol ratio was positively associated with birth weight in women with psychiatric morbidity, also after adjustment for gestational length, fetal sex, maternal height, smoking, SSRI use, and time of blood sampling (standardized β=0.35, p<0.001), with no association in the healthy controls. Thus, the maternal serum cortisone to cortisol ratio does not seem to be affected by psychiatric morbidity, but psychiatric morbidity may increase fetal exposure to cortisol or other metabolic factors influencing fetal growth.


49. Domestication Effects On Stress Induced Steroid Secretion And Adrenal Gene Expression In Chickens

Keywords: Steroids, UPC2

Abstract

Understanding the genetic basis of phenotypic diversity is a challenge in contemporary biology. Domestication provides a model for unravelling aspects of the genetic basis of stress sensitivity. The ancestral Red Junglefowl (RJF) exhibits greater fear-related behaviour and a more pronounced
HPA-axis reactivity than its domesticated counterpart, the White Leghorn (WL). By comparing hormones (plasmatic) and adrenal global gene transcription profiles between WL and RJF in response to an acute stress event, we investigated the molecular basis for the altered physiological stress responsiveness in domesticated chickens. Basal levels of pregnenolone and dehydroepiandrosterone as well as corticosterone response were lower in WL. Microarray analysis of gene expression in adrenal glands showed a significant breed effect in a large number of transcripts with over-representation of genes in the channel activity pathway. The expression of the best-known steroidogenesis genes were similar across the breeds used. Transcription levels of acute stress response genes such as StAR, CH25 and POMC were upregulated in response to acute stress. Dampened HPA reactivity in domesticated chickens was associated with changes in the expression of several genes that presents potentially minor regulatory effects rather than by means of change in expression of critical steroidogenic genes in the adrenal.

50. The Utility Of Ultra High Performance Supercritical Fluid Chromatography For The Analysis Of Seized Drugs: Application To Synthetic Cannabinoids And Bath Salts

Project Purpose
The purpose of this project is to investigate the role of ultra high performance supercritical fluid chromatography (UHPSFC) as a separation technique for forensic drug analysis. For this reason the challenging separation of emerging drugs such as synthetic cannabinoids and bath salts will be investigated. Emerging drugs contain similar solutes such as analogues, homologues, positional isomers, and stereoisomers. The latter two classes of compounds can present particularly difficult separation challenges for which UHPSFC appears well suited. An additional goal of this study is to establish UHPSFC as a viable separation technique (recognized as a Category B test by SWGDRUG) comparable to already established techniques for the separation of emerging drugs. For UHPSFC, validated methods for the determination of synthetic cannabinoids and synthetic cathinones (bath salts) in seized exhibits using UV-PDA detection and single-quad MS detection will be developed.

Project Design
Experiments were designed to answer the question whether UHPSFC is a viable technique for forensic drug analysis, and whether as expected it is particularly well suited, compared to conventional techniques, for the separation of closely related substances (analogues, homologues, positional isomers, and diastereomers), which are present in emerging drug exhibits. The study consisted of three phases. For the first phase, using a UHPSFC instrument equipped with a PDA-UV detector and a single-quad mass spectrometric detector, optimum chromatographic and detector conditions were established for the separation of selected synthetic cannabinoids and substituted cathinones.

51. Comparison Of Ultra High Performance Supercritical Fluid Chromatography, Ultra High Performance Liquid Chromatography, And Gas Chromatography For The Separation Of Synthetic Cathinones

Abstract
A comparison of ultra high performance supercritical fluid chromatography, ultra high performance liquid chromatography, and gas chromatography for the separation of synthetic cathinones has been conducted. Nine different mixtures of bath salts were analyzed in this study. The three different chromatographic techniques were examined using a general set of controlled synthetic cathinones as well as a variety of other synthetic cathinones that exist as positional isomers. Overall 35 different synthetic cathinones were analyzed. A variety of column types and chromatographic modes were examined for developing each separation. For the ultra high performance supercritical fluid chromatography separations, analyses were performed using a series of Torus and Trefoil columns with either ammonium formate or ammonium hydroxide as additives, and methanol, ethanol or isopropanol organic solvents as modifiers. Ultra high performance liquid chromatographic separations were performed in both reversed phase and hydrophilic interaction chromatographic modes using SPP C18 and SPP HILIC columns. Gas chromatography separations were performed using an Elite-5MS capillary column. The
orthogonality of ultra high performance supercritical fluid chromatography, ultra high performance liquid chromatography, and gas chromatography was examined using principal component analysis. For the best overall separation of synthetic cathinones, the use of ultra high performance supercritical fluid chromatography in combination with gas chromatography is recommended. https://doi.org/10.1002/jssc.201700349


Keywords: CuO oxidation, Humic acid, Lignin, Phenolic compounds, Ultra-high performance supercritical fluid chromatography

Abstract
Traditional chromatographic methods for the analysis of lignin-derived phenolic compounds in environmental samples are generally time consuming. In this work, an ultra-high performance supercritical fluid chromatography method with a diode array detector for the analysis of major lignin-derived phenolic compounds produced by alkaline cupric oxide oxidation was developed. In an analysis of a collection of 11 representative monomeric lignin phenolic compounds, all compounds were clearly separated within 6 min with excellent peak shapes, with a limit of detection of 0.5–2.5 μM, a limit of quantification of 2.5–5.0 μM, and a dynamic range of 5.0–2.0 mM ($R^2 > 0.997$). The new ultra-high performance supercritical fluid chromatography method was also applied for the qualitative and quantitative analysis of lignin-derived phenolic compounds obtained upon alkaline cupric oxide oxidation of a commercial humic acid. Ten out of the previous eleven model compounds could be quantified in the oxidized humic acid sample. The high separation power and short analysis time obtained demonstrate for the first time that supercritical fluid chromatography is a fast and reliable technique for the analysis of lignin-derived phenols in complex environmental samples. https://doi.org/10.1002/jssc.201600169

53. Ultra-High-Performance Supercritical Fluid Chromatography as a Separation Tool for Fusarium Mycotoxins and Their Modified Forms 2017 – Journal of AOAC International

Keywords: Beer, NIV, DON, 3-ADON, 15-ADON, DON-3G, NEO, DAS, T-2, HT-2, T-2-3G, ZEN, a-ZEL, and b-ZEL, UPC2, multi-mycotoxin analysis

Abstract
A simple, reliable method for the detection of free and modified Fusarium mycotoxins in beer using state-of-the-art ultra-high-performance supercritical fluid chromatography (UHPSFC) with low-resolution tandem MS (MS/MS) is presented in this paper. The UHPSFC-MS/MS method was developed for nivalenol, deoxynivalenol, 15-acetyl-deoxynivalenol, 3-acetyl-deoxynivalenol, deoxynivalenol-3-glucoside, HT-2 toxin, T-2 toxin, T-2 toxin-3-glucoside, neosolaniol, diacetoxyisocirpenol, zearalenone, α-zearalenol, and β-zearalenol and their internal standards deepoxy-deoxynivalenol and zearalanone. Due to the broad range of the physicochemical properties of the aforementioned, the sample preparation step was minimized to avoid analyte losses. Extraction with acetonitrile–water–acetic acid (79 + 20 + 1, v/v/v) and hexane in combination with solid-phase extraction (C18) was followed by a filtration step. After filtration, the extract was evaporated, and the remaining residue was redissolved in a mobile phase for injection (methanol–water; 90 + 10, v/v). A mobile phase consisting of supercritical CO2 and a small portion of methanol was used. The developed multimycotoxin method permits the simultaneous determination of multiple fusariotoxins in an one-step chromatographic run using UHPSFC-MS/MS. SFC is a promising strategy; however, the retention mechanism is complex, leading to some mycotoxins not being retained on the column. This restricts the applicability of UHPSFC in multimycotoxin analyses. The present study is the first report on the use of UHPSFC for the analysis of free and modified Fusarium mycotoxins. https://doi.org/10.5740/jaoacint.17-0336


Keywords: Tobacco, UPC2
Abstract
Nornicotine, an alkaloid constituent of tobacco, is a precursor to the carcinogen N-nitrosornicotine that is produced during the curing and processing of tobacco. Accumulating evidence reveals that nornicotine enantiomers have different neurochemical and behavioral effects. In the present study, an accurate and rapid method was developed for the enantioseparation of (R)-(+)nornicotine and (S)-(−)nornicotine enantiomers in tobacco by ultraperformance convergence chromatography with tandem mass spectrometry. Chromatographic conditions were investigated to achieve the optimal resolution of two enantiomers. Results indicated that (R)-(+)nornicotine and (S)-(−)nornicotine could be separated within 5 min when ammonium hydroxide was added into the co-solvent, and the best resolution ($R_s = 4.76$) was achieved on an immobilized cellulose tri(S)-(3,5-dichlorophenylcarbamate) chiral stationary phase. The proposed method was validated and was finally applied to analyze the compositions of (R)-(+)nornicotine and (S)-(−)nornicotine in three typical types of tobaccos (flue-cured, burley, and oriental). It was found that, enantiomer fraction of nornicotine (the proportion of (S)-(−)-nornicotine in the nornicotine pool) in burley tobacco samples was relatively high and constant compared with flue-cured and oriental tobaccos. The effective and rapid enantioseparation of nornicotine may help the understanding of alkaloids metabolites in different tobacco varieties and may also benefit pharmacological studies of alkaloid enantiomers.

https://doi.org/10.1002/jssc.201700759

55. Simultaneous Analysis Of Perfluoroalkyl And Polyfluoroalkyl Substances Including Ultrashort-Chain C2 And C3 Compounds In Rain And River Water Samples By Ultra Performance Convergence Chromatography

Keywords: PFAS, Short-chain, Chromatography, Supercritical fluid chromatography (SFC), Trifluoroacetate (TFA)

Abstract
An analytical method using ultra performance convergence chromatography (UPC²) coupled to a tandem mass spectrometer operated in negative electrospray mode was developed to measure perfluoroalkyl and polyfluoroalkyl substances (PFASs) including the ultrashort-chain PFASs (C2-C3). Compared to the existing liquid chromatography tandem mass spectrometry method using an ion exchange column, the new method has a lower detection limit (0.4 pg trifluoroacetate (TFA) on-column), narrower peak width (3–6 s), and a shorter run time (8 min). Using the same method, different classes of PFASs (e.g., perfluoralkyl sulfonates (PFSAs) and perfluorinated carboxylates (PFCAs), perfluorinated phosphonates (PFPA)s and phosphinates (PFPIs), polyfluoroalkyl phosphate diesters (diPAPs)) can be measured in a single analysis. Rain ($n = 2$) and river water ($n = 2$) samples collected in Toronto, ON, were used for method validation and application. Results showed that short-chain PFAS (C2-C7 PFCAs and C4 PFSAs) contributed to over 80% of the detectable PFASs in rain samples and the C2-C3 PFASs alone accounted for over 40% of the total. Reports on environmental levels of these ultrashort-chain PFASs are relatively scarce. Relatively large contribution of these ultrashort-chain PFASs to the total PFASs indicate the need to include the measurement of short-chain PFASs, especially C2 and C3 PFASs, in environmental monitoring. The sources of TFA and other short-chain PFASs in the environment are not entirely clear. The newly developed analytical method may help further investigation on the sources and the environmental levels of these ultrashort-chain PFASs.

https://doi.org/10.1016/j.chroma.2017.09.049

56. Step Economy Strategy For The Synthesis Of Amphoteric Aminoaldehydes, Key Intermediates For Reduced Hydantoins

Keywords: amphoteric aminoaldehydes, aziridination, hydantoin, ICGC-6, organocatalysis, step economy

Abstract
Despite of the orthogonal reactivity of the N–H aziridines aldehyde, these compounds exist as an equilibrium of three different forms – whereas the dimeric one is mostly observed in a variety of solvents. In this work, we have developed an alternative protocol for the aminoaldehyde dimers synthesis in two steps starting with an organocatalyzed aziridination between α,β-unsaturated aldehydes and a protected amine to afford known isolable and stable N-protected aziridine
aldehydes. After Boc-deprotection, dimeric species were immediately formed from monomeric N–H aziridine aldehydes. From this building-block new reduced hydantoins were prepared via [3+2]-
annulation with isocyanates.
https://doi.org/10.1515/pac-2017-0705

57. Supercritical Fluid Chromatography In Traditional Chinese Medicine Analysis
2017 - Journal of Pharmaceutical and Biomedical Analysis
Keywords: Supercritical fluid chromatography, Traditional chinese medicines, Achiral separations
Abstract
Traditional Chinese medicines (TCMs) are gaining increasing popularity throughout the world due to their long historical clinical practices. Highly efficient analytical separation tools are essential for investigating the mysterious properties of TCMs and their quality control. Supercritical fluid chromatography (SFC) showed a great potential in TCM analysis for both nonpolar and polar components. In this paper, an overview of the experimental conditions (i.e. detection mode, stationary phase, mobile phase composition, pressure and temperature) used in SFC for achiral separations of TCM components is presented and recent applications to the analysis of different classes of compounds extracted from TCMs, such as lipids, terpene and terpenoids, phenolic compounds, flavonoids, alkaloids, saponins and carbohydrates, will be briefly described.
https://doi.org/10.1016/j.jpba.2017.08.021

58. Consequences Of Transition From Liquid Chromatography To Supercritical Fluid Chromatography On The Overall Performance Of A Chiral Zwitterionic Ion-Exchanger
2017 - Journal of Chromatography A
Keywords: Enantioseparation, Supercritical fluid chromatography, High performance liquid chromatography, Enhanced-fluidity mobile phase, Amino acids, Transient acid
Abstract
Major differences in the chromatographic performance of a zwitterion ion-exchange type (ZWIX) chiral stationary phase (CSP) in supercritical fluid chromatography (SFC) and high-performance liquid chromatography (HPLC) have been observed. To explain these differences, transition from HPLC to SFC conditions has been performed. The amount of a protic organic modifier in supercritical carbon dioxide (scCO2) was stepwise increased and the effect of this change studied using acidic, basic and ampholytic analytes. At the same time, the effect of various basic additives to the mobile phase and transient acidic buffer species, formed by the reaction of scCO2 with the organic modifier and additives, was assessed. Evidence is provided that a transient acid together with the intrinsic counter-ions present in the ZWIX selector structure drive the elution of analytes even when no buffer is employed. We show that the tested analytes can be enantioseparated under both SFC and HPLC conditions; the best conditions for the resolution of ampholytes are in the so-called enhanced-fluidity mobile phase region. As a consequence, subcritical fluid and enhanced-fluidity mobile phase regions seem to be chromatographic modes with a high potential for operating ZWIX CSPs.
https://doi.org/10.1016/j.chroma.2017.08.022

2017 - Journal of Pharmaceutical and Biomedical Analysis
Keywords: Liquid chromatography, SFC, Mass spectrometry, LC–MS, Medicinal plants, Natural products, Analysis
Abstract
The separation, identification and quantification of constituents in complex plant extracts always has been, and most likely will be, a challenging task. Nevertheless, today a multiplicity of different separation techniques, specific stationary phases and detectors are available, helping to achieve the desired selectivity, sensitivity and speed for nearly any separation problem. The most prominent and popular technique in this area of research is definitely the combination of liquid chromatography and mass spectrometry. More than 40 years after its beginning LC–MS can be considered a well-established routine technique, however there is a steady advancement in terms of instrumentation (ultra-high-performance LC, ion mobility MS, etc.), the hyphenation of different techniques (supercritical fluid chromatography – mass spectrometry, two-dimensional techniques,
etc.), or the type of analyzed compounds (novel applications). The here presented review aims to consider all of these aspects, focusing on natural products/medicinal plants related LC–MS papers published within the years 2011–2016. It gives a short overview of recent technical trends as well summarizes the most relevant applications ordered by the type of natural products assessed (e.g. acids, alkaloids, flavonoids, terpenes). The respective reports are also differentiated according to the studies purpose (analysis of plant material or pharmacological investigation) and a special chapter is devoted to Traditional Chinese Medicine. For selected reports relevant methodological details are provided and limitations or advantages discussed, so that the current status of LC–MS for natural products analysis is reflected comprehensively.

https://doi.org/10.1016/j.jpba.2017.07.038

60. Investigation Of The Effect Of Column Temperature And Back-Pressure In Achiral Supercritical Fluid Chromatography Within The Context Of Drug Impurity Profiling

Keywords: Dissimilar stationary phases, Response surface design, Retention prediction, Separation optimization, Chromatographic efficiency

Abstract

Twenty commercially available stationary phases were characterized in supercritical fluid chromatography (SFC) using a diverse set of pharmaceutical compounds. Six dissimilar phases were selected, and a benzodiazepine and a trimethoprim impurity mixture were screened on these phases. Two stationary phases were then selected for each mixture to study the effect of temperature and back-pressure on retention, separation and chromatographic efficiency using a response surface design approach. The maximal feasible domain for each phase was examined and column performance was monitored for stability during the duration of the study. Chromatographic responses of the individual mixture components, such as retention time, peak width and apparent plate count, were modeled as a function of temperature and back-pressure. The use of high temperatures led to improved separations and higher efficiencies while high back-pressures resulted in reduced retention. For the two mixtures, the response surface plots of the resolution of the worst-separated peak pair over the experimental domain allowed the identification of the temperature and back-pressure leading to the maximal resolution for the worst-separated peak pair. For the mixtures investigated, the use of high temperatures led to improved separations and high efficiencies, while high back-pressures resulted in reduced retention. These factors are fine-tuning parameters in SFC, and similarly to the modifier composition, they lead to local, rather than global selectivity differences.

https://doi.org/10.1016/j.chroma.2017.08.008

61. Determination Of Fat- And Water-Soluble Vitamins By Supercritical Fluid Chromatography: A Review

Keywords: LSER, linear solvation energy relationships, SMB, simulated moving bed, UPC², ultra-performance convergence chromatography
Abstract
Vitamins are compounds that take part in all basic functions of an organism but also are subject of number of studies performed by different researchers. Two groups of vitamins are distinguished taking into consideration their solubility. Chromatography with supercritical CO2 has found application in the determination, separation, and quantitative analyses of both fat- and water-soluble vitamins. The methods of vitamins separation have developed and improved throughout the years. Both groups of compounds were separated using supercritical fluid chromatography with different detection on different stationary phases. The main aim of this review is to provide an overview of the studies of vitamins separation that have been determined so far.

https://doi.org/10.1002/jssc.201700598

62. Is Supercritical Fluid Chromatography Hyphenated To Mass Spectrometry Suitable For The Quality Control Of Vitamin D3 Oily Formulations?
Keywords: SFC-MS, Green analytical chemistry, Vitamin D3, Method validation, Accuracy profile

Abstract
Nowadays, many efforts are devoted to improve analytical methods regarding efficiency, analysis time and greenness. In this context, Supercritical Fluid Chromatography (SFC) is often regarded as a good alternative over Normal Phase Liquid Chromatography (NPLC). Indeed, modern SFC separations are fast, efficient with suitable quantitative performances. Moreover, the hyphenation of SFC to mass spectrometry (MS) provides additional gains in specificity and sensitivity. The present work aims at the determination of vitamin D3 by SFC-MS for routine Quality Control (QC) of medicines specifically. Based on the chromatographic parameters previously defined in SFC-UV by Design of Experiments (DoE) and Design Space methodology, the method was adapted to work under isopycnic conditions ensuring a baseline separation of the compounds. Afterwards, the response provided by the MS detector was optimized by means of DoE methodology associated to desirability functions. Using these optimal MS parameters, quantitative performances of the SFC-MS method were challenged by means of total error approach method validation. The resulting accuracy profile demonstrated the full validity of the SFC-MS method. It was indeed possible to meet the specification established by the European Medicines Agency (EMA) (i.e. 95.0 − 105.0% of the API content) for a dosing range corresponding to at least 70.0-130.0% of the API content. These results highlight the possibility to use SFC-MS for the QC of medicine and obviously support the switch to greener analytical methods.

https://doi.org/10.1016/j.chroma.2017.07.057

63. Ultra-High Performance Supercritical Fluid Chromatography-Mass Spectrometry Procedure For Analysis Of Monosaccharides From Plant Gum Binders
Keywords: Supercritical fluid chromatography, Mass spectrometry, Microwave-assisted hydrolysis, Monosaccharide, Plant gum, Watercolor

Abstract
The ultra-high performance supercritical fluid chromatography-mass spectrometry (UHP-SFC/MS) procedure for analysis of native monosaccharides was developed. Chromatographic conditions were investigated to separate a mixture of four hexoses, three pentoses, two deoxyhexoses and two uronic acids. Increasing water content in methanol modifier to 5% and formic acid to 4% improved peak shapes of neutral monosaccharides and allowed complete elution of highly polar uronic acids in a single run. An Acquity HSS C18SB column outperformed other three tested stationary phases (BEH (silica), BEH 2-ethylpyridine, CSH Fluoro-Phenyl) in terms of separation of isomers and analysis time (4.5 min). Limits of detection were in the range 0.01–0.12 ng μL−1. Owing to separation of anomers, identification of critical pairs (arabinose-xylose and glucose-galactose) was possible. Feasibility of the new method was demonstrated on plant-derived polysaccharide binders. Samples of watercolor paints, painted paper and three plant gums widely encountered in painting media (Arabic, cherry and tragacanth) were decomposed prior the analysis by microwave-assisted hydrolysis at 40 bar initial pressure using 2 mol L−1 trifluoroacetic acid. Among tested temperatures, 120 °C ensured appropriate hydrolysis efficiency for different types of gum and avoided excessive degradation of labile monosaccharides. Procedure recovery tested on gum Arabic was 101% with an RSD below 8%. Aqueous hydrolysates containing monosaccharides in different ratios specific to each type of plant gum were diluted or analyzed.
directly. Filtration of samples before hydrolysis reduced interferences from a paper support and identification of gum Arabic in watercolor-painted paper samples was demonstrated. Successful identification of pure gum Arabic was confirmed for sample quantities as little as 1 μg. Two classification approaches were compared and principal component analysis was superior to analysis based on peak area ratios of monosaccharides. The proposed procedure using UHPSFC/MS represents an interesting alternative which can compete with other chromatographic methods in the field of saccharide analysis in terms of speed, sensitivity and simplicity of workflow.

https://doi.org/10.1016/j.aca.2017.07.036

64. Development And Comparison Of Quantitative Methods Using Orthogonal Chromatographic Techniques For The Analysis Of Potential Mutagenic Impurities

Introduction
There are many steps during the manufacturing process of an active pharmaceutical ingredient (API) where impurities can be introduced, whether as reagents, byproducts, intermediates, etc. Some of these impurities may be mutagenic, or have the potential to interact with DNA and ultimately cause carcinogenicity. Methodologies associated with monitoring API purity levels are often HPLC-UV based, which frequently do not provide the sensitivity levels needed to detect potentially mutagenic impurities (PMIs) at the levels required by regulatory agencies. For example, ondansetron is a pharmaceutical used in the prevention of nausea and vomiting and may contain one potential mutagenic impurity, 2-methylimidazole, as well as a second impurity very closely related in structure, imidazole.

Similar to 2-methylimidazole and imidazole, many mutagenic impurities are small, highly polar compounds that are poorly retained under typical reversed phase liquid chromatography (RPLC) conditions. Alternate forms of chromatography, such as hydrophilic interaction chromatography (HILIC), or the use of ion-pairing reagents can be employed, but these often result in tedious method development or non-MS friendly mobile phases. Supercritical fluid chromatography (SFC) is known to be orthogonal to RPLC, and employs reagents which are suitable for MS detection. In this study, methods for the analysis of ondansetron and five organic impurities were developed using both liquid and supercritical fluid chromatographic methods. Both chromatographic techniques generated high sensitivity methods that met the required limits of detection and both techniques showed good accuracy and reproducibility.
65. Enantiomeric Separation And Quantification Of Citalopram In Serum By Ultra-High Performance Supercritical Fluid Chromatography-Tandem Mass Spectrometry

Keywords: UHPSFC-MS/MS, Citalopram, Enantiomeric, Serum, Therapeutic drug monitoring

Abstract
A method for enantiomeric separation and quantification of R/S-citalopram in serum was developed and validated using ultra-high performance supercritical fluid chromatography-tandem mass spectrometry (UHPSFC-MS/MS). Sample preparation prior to UHPSFC-MS/MS analysis consisted of protein precipitation with acidic acetonitrile and filtration through a phospholipid removal plate. The UHPSFC-MS/MS method used an UPC² Trefoil CEL2 column with a mobile phase consisting of CO₂ and methanol/acetonitrile (70:30, v/v) with 10 mM ammonium acetate. The injection volume was 1 μL and run time was 4 min. MS/MS detection was performed with positive electrospray ionization and two multiple reaction monitoring transitions (m/z 325.1 > 262.0 and m/z325.1 > 109.0). The calibration range was 5–500 nM for each analyte. The between-assay relative standard deviations were in the range of 3.4–4.5%. Recovery was 81–91% and matrix effects ranged from 96 to 101% (corrected with internal standard). After development and initial testing, the method has been successfully implemented in routine use in our laboratory for both separation and quantification of R/S-citalopram in more than 250 serum samples for therapeutic drug monitoring.

https://doi.org/10.1016/j.jchromb.2017.07.009

66. Coupling Supercritical Fluid Chromatography To Positive Ion Atmospheric Pressure Ionization Mass Spectrometry: Ionization Optimization Of Halogenated Environmental Contaminants

Keywords: SFC, Positive APPI-MS, Environmental contaminants, PCDD, PCDF, PCB

Abstract
Currently used analytical techniques for halogenated aromatic environmental contaminants such as polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs), also known as legacy persistent organic pollutants, are based on gas chromatographic separation of target analytes and detection by mass spectrometry. The coupling of packed column supercritical fluid chromatography (SFC) to atmospheric pressure ionization mass spectrometry (API/MS) could allow for the concurrent analysis of thermally labile and legacy halogenated environmental contaminants if ionization can be sufficiently optimized. The evaluation of positive ion atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) as well as possible charge transfer dopants for the generation of molecular ion isotopomeric clusters of halogenated environmental contaminants with minimal fragmentation has been completed. Using the investigated parameters, positive ion APPI was found to be the more sensitive technique. Of the aromatic and cycloalkane dopants investigated, only fluorobenzene and trifluorotoluene were found to be effective dopants for the halogenated aromatic target analytes (PCDDs, PCDFs, and PCBs). Experiments involving deuterated dopants confirmed that reactive species generated by cycloalkanes were quenched by the SFC eluent rendering them unusable in conjunction with the investigated separation technique. Alternatively, aromatic dopants were found to be less susceptible to quenching by the SFC eluent and fluorobenzene was determined to be the most effective charge transfer dopant for PCDDs, PCDFs, and PCBs. To demonstrate the applicability of the optimized ionization conditions, SFC-API/MS has been used for the concurrent analysis of legacy halogenated aromatic environmental contaminants (PCDDs, PCDFs, and PCBs) and thermally labile analytes (α, β, and γ isomers of hexabromocyclododecane).

https://doi.org/10.1016/j.ijms.2017.07.005
Analysis Of Oxylipins In Human Plasma: Comparison Of Ultrahigh-Performance Liquid Chromatography And Ultrahigh-Performance Supercritical Fluid Chromatography Coupled To Mass Spectrometry

Keywords: Oxylipin, Eicosanoid, Prostaglandin, Plasma, UHPSFC/MS, UHPLC/MS, SPE, ESI

Abstract

The potential of ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC/MS) and ultrahigh-performance supercritical fluid chromatography (UHPSFC) coupled to negative-ion electrospray ionization mass spectrometry (ESI–MS) for the analysis of 46 oxylipins and 2 fatty acid standards is compared in terms of their chromatographic resolution with the emphasis on distinguishing isobaric interferences and the method sensitivity. UHPLC provides the baseline separation of 24 isobaric oxylipins within 13 min, while UHPSFC enables the separation of only 20 isobaric oxylipins within 8 min. Moreover, the UHPLC/ESI–MS method provides an average improvement of sensitivity by 3.5-fold. A similar trend is observed in the analysis of human plasma samples, but lower ion suppression effects caused by lysophospholipids (LPL) are observed in case of UHPSFC/ESI–MS due to better separation of LPL. Both methods are fully applicable for the analysis of oxylipins, but UHPLC/ESI–MS method is preferred due to better separation and higher sensitivity, which results in the identification of 31 oxylipins in human plasma based on available standards and additional tentative 20 identifications based on accurate m/z values and the fragmentation behavior known from the literature.

https://doi.org/10.1016/j.chroma.2017.06.070

A Systematic Investigation Of Sample Diluents In Modern Supercritical Fluid Chromatography

Keywords: Supercritical fluid chromatography, Sample diluent, Peak shape, UHPSFC, Injection

Abstract

This paper focuses on the possibility to inject large volumes (up to 10 μL) in ultra-high performance supercritical fluid chromatography (UHPSFC) under generic gradient conditions. Several injection and method parameters have been individually evaluated (i.e. analyte concentration, injection volume, initial percentage of co-solvent in the gradient, nature of the weak needle wash solvent, nature of the sample diluent, nature of the column and of the analyte). The most critical parameters were further investigated using in a multivariate approach. The overall results suggested that several aprotic solvents including methyl tert-butyl ether (MTBE), dichloromethane, acetonitrile or cyclopentyl methyl ether (CPME) were well adapted for the injection of large volume in UHPSFC, while MeOH was generally the worst alternative. However, the nature of the stationary phase also had a strong impact and some of these diluents did not perform equally on each column. This was due to the existence of a competition in the adsorption of the analyte and the diluent on the stationary phase. This observation introduced the idea that the sample diluent should not only be chosen according to the analyte but also to the column chemistry to limit the interactions between the diluent and the ligands. Other important
characteristics of the “ideal” SFC sample diluent were finally highlighted. Aprotic solvents with low viscosity are preferable to avoid strong solvent effects and viscous fingering, respectively. In the end, the authors suggest that the choice of the sample diluent should be part of the method development, as a function of the analyte and the selected stationary phase.

https://doi.org/10.1016/j.chroma.2017.06.075

69. The Effect Of High Concentration Additive On Chiral Separations In Supercritical Fluid Chromatography

Keywords: Supercritical fluid chromatography, Polysaccharide stationary phases, Enantiomer elution order reversal, High concentration of additive

Abstract
Supercritical Fluid Chromatography is frequently used to efficiently handle separations of enantiomers. The separation of basic analytes usually requires the addition of a basic additive in the mobile phase to improve the peak shape or even to elute the compounds. The effect of increasing the concentration of 2-propylamine as additive on the elution of a series of basic compounds on a Chiralpak-AD stationary phase was studied. In this study, unusual additive concentrations ranging from 0.3% to 10% of 2-propylamine 2-propylamine in the modifier were explored and the effect on retention, peak shape, selectivity and resolution was evaluated. The addition of a large quantity of additive allowed to drastically improve the selectivity and the resolution, and even enantiomers elution order reversal was observed by changing the concentration of basic additive. The role of the ratio additive/modifier appeared a key to tune the enantioselectivity. Finally, the impact of these drastic conditions on the column material was evaluated.

https://doi.org/10.1016/j.chroma.2017.06.049

70. Separation Of Furostanol Saponins By Supercritical Fluid Chromatography

Keywords: Chromatographic behavior, Furostanol saponin, Methoxylation, Supercritical fluid chromatography

Abstract
Supercritical fluid chromatography (SFC) has good separation efficiency and is suitable for separating weakly polar compounds. Furostanol saponins, as an important kind of steroidal saponins, generally have two sugar chains, which are polar and hydrophilic. The hydroxyl group at the C-22 position of furostanol saponins is active and easily reacts with lower alcohols under appropriate conditions. The separation of hydrophilic furostanol saponins was tested by SFC in this study. The effects of chromatographic conditions on the separation of the mixed furostanol saponins and their hydroxyl derivatives at the C-22 position were studied. The conditions for SFC, which included different column polarity, modifier, additive, and column temperature, were tested. After optimization, the mixed 10 similar structures of furostanol saponins were separated in 22 min on the Diol column at a temperature of 40 °C. The mobile phase was CO$_2$ (mobile phase A) and methanol (containing 0.2% NH$_3$-H$_2$O and 3% H$_2$O) (mobile phase B). The backpressure was maintained isobarically at 11.03 MPa. SFC was found to be effective in separating the furostanol saponins that shared the same aglycone but varied in sugar chains. SFC was sensitive to the number and type of sugars. The resolution of furostanol saponin isomers was not ideal. The extract of Dioscorea zingiberensis C. H. Wright was profiled by SFC–quadrupole time-of-flight mass spectrometry. The main saponins of the extract were well separated. Therefore, SFC could be used for separating hydrophilic furostanol saponins and analyzing traditional Chinese medicines that mainly contained steroidal saponins.

https://doi.org/10.1016/j.jpba.2017.05.023
71. Purification Of Lignans From *Fructus Arctii* Using Off-Line Two-Dimensional Supercritical Fluid Chromatography/Reversed-Phase Liquid Chromatography

Keywords: lignan, reversed-phase liquid chromatography, supercritical fluid chromatography, traditional Chinese medicine, two-dimensional chromatography

**Abstract**

As a common traditional Chinese medicine, *Fructus Arctii* has important clinical medical values. Its main components are lignans, which are difficult to separate and analyze because of the complex composition, similar chemical structures, and close properties. In this study, an off-line two-dimensional supercritical fluid chromatography/reversed-phase liquid chromatography method, as well as an effective sample pretreatment method based on hydrophilic interaction chromatography material, was developed to enrich the minor lignan fractions and obtain high-purity compounds. In total, 12 high-purity compounds were isolated from *Fructus Arctii*. Their structures were identified by using high-resolution mass spectrometry and nuclear magnetic resonance spectroscopy, which showed that all were lignans and that most of them were isomers. The results demonstrated the effective off-line two-dimensional supercritical fluid chromatography/reversed-phase liquid chromatography method for the purification of lignans from *Fructus Arctii*. The separation protocol established here will be beneficial for the separation of complex samples from other kinds of natural products.

[https://doi.org/10.1002/jssc.201700139](https://doi.org/10.1002/jssc.201700139)

72. Rapid Purification Of Diastereoisomers From *Piper Kadsura* Using Supercritical Fluid Chromatography With Chiral Stationary Phases

Keywords: Chiral supercritical fluid chromatography, Achiral separation, Diastereoisomers, Purification, *Piper kadsura*

**Abstract**

Supercritical fluid chromatography (SFC) with chiral stationary phases (CSPs) is an advanced solution for the separation of achiral compounds in *Piper kadsura*. Analogues and stereoisomers are abundant in natural products, but there are obstacles in separation using conventional method. In this paper, four lignan diastereoisomers, (-)-Galbelgin, (-)-Ganschisandrin, Galgravin and (-)-Veraguensin, from *Piper kadsura* were separated and purified by chiral SFC. Purification strategy was designed, considering of the compound enrichment, sample purity and purification throughput. Two-step achiral purification method on chiral preparative columns with stacked automated injections was developed. Unconventional mobile phase modifier dichloromethane (DCM) was applied to improve the sample solubility. Four diastereoisomers was prepared at the respective weight of 103.1 mg, 10.0 mg, 152.3 mg and 178.6 mg from 710 mg extract with the purity of greater than 98%.

[https://doi.org/10.1016/j.chroma.2017.06.020](https://doi.org/10.1016/j.chroma.2017.06.020)
73. Rapid Determination Of 9 Aromatic Amines In Mainstream Cigarette Smoke By Modified Dispersive Liquid Liquid Microextraction And Ultraperformance Convergence Chromatography Tandem Mass Spectrometry

Keywords: Aromatic amines, Dispersive liquid liquid microextraction, Ultraperformance convergence chromatography tandem mass spectrometry, Mainstream cigarette smoke

Abstract
Aromatic amines in mainstream cigarette smoke have long been monitored due to their carcinogenic toxicity. In this work, a reliable and rapid method was developed for the simultaneous determination of 9 aromatic amines in mainstream cigarette smoke by modified dispersive liquid liquid microextraction (DLLME) and ultraperformance convergence chromatography tandem mass spectrometry (UPC²-MS/MS). Briefly, the particulate phase of the cigarette smoke was captured by a Cambridge filter pad, and diluted hydrogen chloride aqueous solution is employed to extract the aromatic amines under mechanical shaking. After alkalization with sodium hydroxide solution, small amount of toluene was introduced to further extract and enrich aromatic amines by modified DLLME under vortexing. After centrifugation, toluene phase was purified by a universal QuEChERS cleanup kit and was finally analyzed by UPC²-MS/MS. Attributing to the superior performance of UPC²-MS/MS, this novel approach allowed the separation and determination of 9 aromatic amines within 5.0 min with satisfactory resolution and sensitivity. The proposed method was finally validated using Kentucky reference cigarette 3R4F, and emission levels of targeted aromatic amines determined were comparable to previously reported methods. At three different spiked levels, the recoveries of most analytes were ranged from 74.01% to 120.50% with relative standard deviation (RSD) less than 12%, except that the recovery of \( p \)-toluidine at low spiked level and 3-aminobiphenyl at medium spiked level was 62.77% and 69.37% respectively. Thus, this work provides a novel alternative method for the simultaneous analysis of 9 aromatic amines in mainstream cigarette smoke.

https://doi.org/10.1016/j.chroma.2017.05.056


Keywords: Supercritical fluid chromatography, Mass spectrometry, Bio-oil, Fast pyrolysis, Complex sample

Abstract
The characterization of complex mixtures is a challenging issue for the development of innovative processes dedicated to biofuels and bio-products production. The huge number of compounds present in biomass fast pyrolysis oils combined with the large diversity of chemical functions represent a bottleneck as regards analytical technique development. For the extensive characterization of complex samples, supercritical fluid chromatography (SFC) can be alternative to usual separation techniques such as gas (GC) or liquid chromatography (LC). In this study, an approach is proposed to define the best conditions for the SFC separation of a fast pyrolysis bio-oil. This approach was based on SFC data obtained directly from the bio-oil itself instead of selecting model compounds as usually done. SFC conditions were optimized by using three specific, easy-to-use and quantitative criteria aiming at maximizing the separation power. Polar stationary phases (ethylpyridine bonded silica) associated to a mix of acetonitrile and water as polarity modifier provided the best results, with more than 120 peaks detected in SFC-UV.

https://doi.org/10.1016/j.chroma.2017.06.003

75. Routine Supercritical Fluid Chromatography Tandem Mass Spectrometry Method For Determination Of Vitamin K1 Extracted From Serum With A 96-Well Solid-Phase Extraction Method

Keywords: LC, liquid chromatography; SFC, supercritical fluid chromatography; SFC-MS/MS, supercritical fluid chromatography–tandem mass spectrometry; CRM, certified reference material; DWP, deep-well plate; SPE, solid-phase extraction; BS, bovine serum; KEQAS, external quality
assurance scheme for phylloquinone; PPT, protein precipitation; RLH, robotic liquid handler; UPC2, Ultra Performance Convergence Chromatography; ESI, electrospray ionization

Abstract
With the developed method, measurement of vitamin K1 level in patient serum samples can be performed rapidly and reliably. The method includes a semi-automated solid-phase extraction sample preparation method without the need of an evaporation step before quantification by supercritical fluid chromatography–tandem mass spectrometry (SFC-MS/MS). The method provides a faster analysis than that of LC-MS/MS, and in a period of 3 months, the method has been applied to analysis of >5000 samples. The method is suitable for use in high-throughput medical laboratories. The method allows high-throughput reliable determination of vitamin K1 in serum in the range 0.1–50 ng/mL.

http://jalm.aaccjnls.org/content/jalm/early/2017/03/22/jalm.2016.021717.full.pdf

76. General Screening And Optimization Strategy For Fast Chiral Separations In Modern Supercritical Fluid Chromatography

2017 - Analytica Chimica Acta

Keywords: Supercritical fluid chromatography, SFC, Chiral stationary phases, Organic modifier, Combined additive, Enantioselectivity, Pharmaceuticals

Abstract
High throughput general chiral screening method using supercritical fluid chromatography was developed. This method takes an advantage of very fast gradient screening (3 min + 1 min isocratic hold) and generic enantioselectivity of the combined additive formed by 0.1% trifluoroacetic (TFA) acid and 0.1% diethylamine (DEA). The TFA/DEA combined additive was systematically added to organic modifiers methanol and isopropanol. Among five tested polysaccharide-based chiral stationary phases, amylose tris(3,5-dimethylphenylcarbamate) and cellulose tris(3,5-dimethylphenylcarbamate) provided the best enantioseparation success rate. Therefore, the proposed initial first-line screening includes four experiments using these two stationary phases and the above mentioned two combinations: CO2/methanol and CO2/isopropanol + the combined additive. If these stationary phases fail in the screening step, cellulose tris(3-chloro-4-methylphenylcarbamate) and cellulose tris(3,5-dichlorophenylcarbamate) can be proposed for the screening in the second line.
For further optimization in case of insufficient resolution obtained in the screening phase fine tuning of temperature, BPR pressure and gradient slope was tested with unsuccessful results. An improvement of enantioselectivity was obtained only when gradient elution was replaced by isocratic elution with substantially lower amount of organic modifier, when changing the concentration of the additive or when using combined organic modifier, such as methanol/acetonitrile (1:1). Finally, to enable the MS compatibility, also volatile additives including ammonium formate and ammonium acetate were tested. The results were more encouraging than expected. Volatile buffers thus make an interesting option in chiral SFC screening methods, however, at the cost of somewhat lower enantioselectivity.

http://dx.doi.org/10.1016/j.aca.2016.11.002
77. **New Insights Into Supercritical Fluid Chromatography For Chiral Separations**

2017 - Analytical Methods

**Abstract**

Bioanalysis of stereoisomers represents a great challenge, as chromatographic separation is not feasible using conventional reversed or normal-phase chromatography. Supercritical fluid chromatography in combination with chiral stationary phases has proven to be a great tool for chiral resolution, alleviating some of the challenges associated with bioanalysis of stereoisomers. This technical note describes an alternative to the traditional SFC instrument configuration without the use of a back pressure regulator (BPR) and makeup pump; thereby simplifying its usage and improving the robustness and quality of measurements. This alternative configuration has been successfully employed in our laboratory for multiple chiral applications; in particular for the separation of \textit{cis}/\textit{trans} geometric stereoisomers. Four case studies are presented – one of which was validated demonstrating the robustness of the technology for bioanalytical applications.

[http://dx.doi.org/10.1039/C7AY00452D](http://dx.doi.org/10.1039/C7AY00452D)

78. **The Application Of Green Solvents In Separation Processes - Chapter 16 – Supercritical Fluid Chromatography**
Keywords: Supercritical carbon dioxide; supercritical fluid chromatography; mass spectrometry; lipidomics; unified chromatography

Abstract
Supercritical fluid chromatography (SFC) is a separation technique that characteristically a hybrid of both gas chromatography (GC) and liquid chromatography (LC). With the favorable properties of green solvent supercritical carbon dioxide (SCCO₂) as the main mobile phase, SFC has gained much attraction for analyzing and separating a wide range of compounds. Having remarkable sensitivity and selectivity, mass spectrometer (MS) is widely hyphenated to SFC system. SFC coupled to MS (SFC/MS) approach offers comprehensive lipid analysis that has not been resolved due to the complexity of lipid constituents and the existing isomers. Therefore, SFC/MS has more potential for application in lipidomics field compared to other analytical platforms. The prowess of SFC/MS technology as integral part of green chemistry has also been demonstrated in various fields from food science to biomedical research.

https://doi.org/10.1016/B978-0-12-805297-6.00016-4

79. Plant Lipidomics At The Crossroads: From Technology To Biology Driven Science

Keywords: Plant, lipidomics

Abstract
The identification and quantification of lipids from plant tissues have become commonplace and many researchers now incorporate lipidomics approaches into their experimental studies. Plant lipidomics research continues to involve technological developments such as those in mass spectrometry imaging, but in large part, lipidomics approaches have matured to the point of being accessible to the novice. Here we review some important considerations for those planning to apply plant lipidomics to their biological questions, and offer suggestions for appropriate tools and practices. This article is part of a Special Issue entitled: BBALIP_Lipidomics Opinion Articles edited by Sepp Kohlwein.

https://doi.org/10.1016/j.bbalip.2017.02.011

80. Examination Of Technical Mixtures Of Halogen-Free Phosphorus Based Flame Retardants Using Multiple Analytical Techniques

Keywords: Organophosphate flame retardants (OPFRs); pSFC/MS method; NMR; RDBPP; BPA-BDPP; DOPO

Abstract
Technical RDBPP, BPA-BDPP and DOPO mixtures were characterized using NMR. pSFC/MS enabled fast separation and analysis of all OPFRs and impurities. RDBPP, BPA-BDPP and DOPO mixtures were analyzed by multiple analytical techniques. The application of phosphorus based flame retardants as replacements for commonly used halogenated flame retardants has been gaining interest due to the possibility that these compounds may have a less significant impact on human and environmental health. Unfortunately, little is known about the chemical compositions of many of the technical products (which often are mixtures) and a single separation technique for concurrent analysis of these types of compounds has not been identified. This paper reports the results of an investigation into the constituents of three halogen free organophosphate flame retardants (OPFRs), resorcinol bis(diphenyl phosphate) (RDBPP), bisphenol A bis(diphenyl phosphate) (BPA-BDPP), and 9,10-dihydro-9-oxa-10-phosphaphenanthrene-10-oxide (DOPO). The major components of commercial samples of RDBPP and BPA-BDPP were isolated by preparative TLC and characterized by NMR. A
commercial sample of DOPO was found to be essentially pure, but its analysis is complicated by the fact that it can exist in ring-open and ring-closed forms. With the structures of the components confirmed by NMR, multiple analytical separation techniques (gas chromatography (GC), liquid chromatography (LC), and packed column supercritical fluid chromatography (pSFC)) were investigated for the analysis of these three technical products. Packed column supercritical fluid chromatography allows the separation of the components of all three OPFRs, including the two forms of DOPO, in a single run.

https://doi.org/10.1016/j.chemosphere.2017.02.129

81. Enantioseparation of Chiral Sulfoxides on Amylose-Based Columns: Comparison of Normal Phase Liquid Chromatography and Supercritical Fluid Chromatography

2017 - Chromatographia

Keywords: Enantioseparation; Sulfoxides; Polysaccharide chiral stationary phases; Amylose; Supercritical fluid chromatography; Liquid chromatography

Abstract

We present enantioseparation of a series of racemic sulfoxides on three different amylose-based polysaccharide columns. Two of them have the amylose units modified with dimethylphenyl carbamoyl groups (Chiralpak AD-H and Chiralpak IA), while the third one possesses a carbamoyl moiety with an additional chiral centre (Chiralpak AS-H). The enantioseparation of selected analytes was achieved in high-performance liquid chromatography (HPLC) and the full analyte set was enantiomerically resolved using supercritical fluid chromatography (SFC). Comparing the results obtained in both modes, we show that enantioseparation under SFC conditions is superior to HPLC mode in terms of speed, while retaining excellent enantioselectivity and resolution. Faster elution of analytes was observed on increasing the polarity of the co-solvent (modifier) in the mobile phase. This trend is apparent in both chromatographic modes. Documenting the important role of the additional chiral centre, Chiralpak AS-H provided the best chromatographic parameters resulting in the enantioseparation of all analytes.

82. The Role Of Diode Array Ultraviolet Detection For The Identification Of Synthetic Cathinones

Keywords: diode array; UV detection; UHPSFC; synthetic cathinones

Abstract
The utility of diode array UV detection for aiding in the identification of synthetic cathinones, including different sub classes and positional isomers is presented. For 35 synthetic cathinones’ unique UV spectra are obtained for seven sub classes including mostly beta ketones, where position and type of substitution on benzene rings give rise to differences in UV maxima and relative intensity of the spectral bands. This aspect is key to distinguishing positional isomers which contain differences in R substitution (mono and di) around the benzene ring, which provides complementary information to electron ionization mass spectrometry, where the latter technique cannot distinguish between these types of positional isomers. In addition it is possible to ascertain the substitution position based on the UV spectra. For ten sets of positional isomers it was possible to distinguish most of the positional isomers within a set. For ultra high performance supercritical fluid chromatography (UHPSFC) vs reversed phase ultra-high performance liquid chromatography (UHPLC) there was at least a 10 nm blue shift in UV maximum (shift to shorter wavelengths). This highlights the importance of taking in account the effect of mobile phase on the UV maximum when performing method development in UHPSFC.


83. Selection Of The HPLC Method In Chemical Analysis - Chapter 3 – Short Overviews Of The Main Analytical Techniques Containing A Separation Step

Keywords: Electrochromatography; Electrophoresis; Gas chromatography; HPLC classification; Separation; Supercritical fluid chromatography

Abstract
This chapter presents the main analytical techniques containing a separation step with the goal of online delivering of the separated sample components to a detection device. These techniques can be used as core analytical operations applied for separation and measurement of the components of complex samples. They include chromatographic techniques, solid phase extraction, electroseparations, and membrane separations. Presentation of the main chromatographic separations is also included here: gas chromatography (GC), supercritical fluid chromatography (SFC), high-performance liquid chromatography (HPLC), electrophoresis, and electrochromatography. Selections between various chromatographic techniques with the
description of advantages and disadvantages in selecting HPLC as a method of analysis versus GC, SFC, or zone electrophoresis (CZE) are discussed.

84. Triacylglycerol Compositions Of Sunflower, Corn And Soybean Oils Examined With Supercritical CO2 Ultra-Performance Convergence Chromatography Combined With Quadrupole Time-Of-Flight Mass Spectrometry

Keywords: Triacylglycerol, Sunflower oil, Corn oil, Soybean oil, Ultra-performance convergence chromatography (UPC²), Quadrupole time-of-flight mass spectrometry (Q-TOF MS)

Abstract
A supercritical CO₂ ultra-performance convergence chromatography (UPC²) system was utilized with a quadrupole time-of-flight mass spectrometry (Q-TOF MS) to examine the triacylglycerol compositions of sunflower, corn and soybean oils. UPC² provided an excellent resolution and separation for the triacylglycerols, while the high performance Q-TOF MS system was able to provide the molecular weight and fragment ions information for triacylglycerol compound characterization. A total of 33 triacylglycerols were identified based on their elementary compositions and MS² fragment ion profiles, and their levels in the three oils were estimated. The combination of UPC² and Q-TOF MS may determine triacylglycerol compositions for oils and fats, and provide sn-position information for fatty acids, which may be important for food nutritional value and stability.

85. Enantioseparation Of Methamphetamine By Supercritical Fluid Chromatography With Cellulose- Based Packed Column

Keywords: Supercritical fluid chromatography; Enantiomer; Methamphetamine

Abstract
The enantiomers of methamphetamine were differentiated by supercritical fluid chromatography (SFC) with an enantioselective cellulose-based packed column. The optimization of the chromatographic conditions was achieved by changing column temperature, co-solvent proportion, additive concentration, flow rate and back pressure. In particular, the additive concentration crucially changed the resolution between the enantiomers. After determining the optimized conditions, the enantiomers of methamphetamine were successfully separated. The analytical precision, accuracy and limit of detection were checked by using the authentic standard and seized real samples. We believe that chiral SFC is a promising method for enantioseparation of forensic samples.
86. Physicochemical Properties Of *Acer Truncatum* Seed Oil Extracted Using Supercritical Carbon Dioxide

2017 - Journal of the American Oil Chemists Society

Keywords: Acer truncatum seed oil, Nervonic acid, UPC2-Q-TOF-MS analysis, sn-2, Nutraceutical constituents, Physicochemical properties

Abstract

*Acer truncatum* seed oil rich in nervonic acid was extracted using supercritical carbon dioxide. GC (Gas Chromatography) analysis revealed that the oil contained approximately 6.22% nervonic acid. The *sn*-2 compositions were also determined using lipase hydrolysis. A total of 52 triacylglycerides (TAG) were tentatively identified in the oil using an ultra-performance convergence chromatography (UPC2) coupled with quadrupole time-of-flight mass spectrometry (Q-TOF-MS) for the first time. In addition, the contents of phytosterols (1961.9–2402.8 μmol/kg) and β-carotene (2.09–2.35 μmol/kg) were also quantified for the first time, along with tocopherols (2352.0–2654.3 μmol/kg). The γ-tocopherol (1296.9–1442.3 μmol/kg) was the primary tocopherol, while β-sitosterol (1355.2–1631.3 μmol/kg) was the dominant phytosterol. The physicochemical properties of the oil were also investigated. This study indicated that *A. truncatum* seed oil is rich in nervonic acid and other nutraceutical constituents. It has a high potential in functional foods for improving human health.

http://dx.doi.org/10.1007/s11746-017-2983-1

87. Preparative Supercritical Fluid Chromatography: A Powerful Tool For Chiral Separations

2016 - Journal of Chromatography A

Keywords: Research Department Janssen France, Univ. Lille, Pharmaceutical compounds, Chirality, Supercritical fluid chromatography, Preparative scale, Review

Abstract:

In 2012, the 4 biggest pharmaceutical blockbusters were pure enantiomers and separating racemic mixtures is now frequently a key step in the development of a new drug. For a long time, preparative liquid chromatography was the technique of choice for the separation of chiral compounds either during the drug discovery process to get up to a hundred grams of a pure enantiomer or during the clinical trial phases needing kilograms of material. However the advent of supercritical Fluid Chromatography (SFC) in the 1990s has changed things. Indeed, the use of carbon dioxide as the mobile phase in SFC offers many advantages including high flow rate, short equilibration time as well as low solvent consumption. Despite some initial teething troubles, SFC is becoming the primary method for preparative chiral chromatography. This article will cover recent developments in preparative SFC for the separation of enantiomers, reviewing several
aspects such as instrumentation, chiral stationary phases, mobile phases or purely preparative considerations including overloading, productivity or large scale chromatography.

http://dx.doi.org/10.1016/j.chroma.2016.07.050

88. Scaling Rule In SFC. II. A Practical Rule For Isocratic Systems

Keywords: Supercritical fluid chromatography; Method-transfer; Pressure-drop; Density variation; Density modulation; Preparative; Scaling; Scale-up

Abstract:
Scaling methods, either from analytical to analytical systems or from analytical to preparative systems and vice versa, are commonly performed in chromatography. For liquid chromatography there exist geometric rules for scaling, which provide guidelines to select column dimensions, particle sizes and flow rates. For SFC, on the other hand, there are no such rules or any well-understood principles behind scaling. In a recent report [1] this issue was addressed, proposing a rule of maintaining the same average density in the target system as it was in the original system. The problem with the criterion of maintaining average density, however, is the availability of density data. Not only one needs access and relevant experience of working with physical property data, in many cases density data may not be available at all. The current report demonstrates that a simpler approach, of matching average pressures, is equally applicable for acceptable scaling over most of the operating conditions used in SFC.

http://dx.doi.org/10.1016/j.chroma.2016.12.044

89. Optimization And Validation Of A Fast Supercritical Fluid Chromatography Method For The Quantitative Determination Of Vitamin D3 And Its Related Impurities

Keywords: SFC; Green analytical chemistry; Vitamin D3; Method validation; Accuracy profile; Design space

Abstract
In the uprising context of green analytical chemistry, Supercritical Fluid Chromatography (SFC) is often suggested as an alternative to Normal Phase Liquid Chromatography. Indeed, SFC provides fast, efficient and green separations. In this report, the quantitative performances of SFC were challenged on a real-life case study: the Quality Control (QC) of vitamin D3. A rapid and green SFC method was optimized thanks to the Design of Experiments–Design Space (DoE–DS) methodology. It provided robust and high quality separation of the compounds within a 2 min timeframe, using a gradient of ethanol as co-solvent of the carbon dioxide. The analytical method was fully validated according to the total error approach, demonstrating the compliance of the method to the specifications of U.S. Pharmacopeia (USP: 97.0–103.0%) and European Pharmacopeia (EP: 97.0–102.0%) for an interval of [50–150%] of the target concentration. In order to allow quantification of impurities using vitamin D3 as an external standard in SFC-UV, correction factors were determined and verified during method validation. Thus, accurate quantification of impurities was demonstrated at the specified levels (0.1 and 1.0% of the main compound) for a 70.0–130.0% dosing range. This work demonstrates the validity of an SFC method for the QC of vitamin D3 raw material and its application to real samples. Therefore, it supports the switch to a greener and faster separative technique as an alternative to NPLC in the pharmaceutical industry.

http://dx.doi.org/10.1016/j.chroma.2017.01.090

90. Unravelling The Effects Of Mobile Phase Additives In Supercritical Fluid Chromatography. Part I: Polarity And Acidity Of The Mobile Phase

Keywords: Additives, Colour indicators, Mobile phase acidity, Solvatochromic dye, Supercritical fluid chromatography

Abstract
Polarity effects of mobile phase additives are assessed with a solvatochromic probe. Additives in usual concentration ranges cause no significant change in polarity. Acidity of SFC mobile phases is assessed with pH colour indicators.
Apparent pH in carbon dioxide-methanol mixtures is around 5.

Basic additives do not yield basic conditions, acidic additives yield acidic conditions. The mobile phases employed in current supercritical fluid chromatography (SFC) are usually composed of a mixture of pressurized carbon dioxide and a co-solvent. The co-solvent is most often an alcohol and may contain a third component in small proportions, called an additive (acid, base or salt). The polarity of such mobile phase compositions is here re-evaluated with a solvatochromic dye (Nile Red), particularly to assess the contribution of additives. It appears that additives, when employed in usual concentration range (0.1% or 20 mM) do not modify the polarity in the immediate environment of the probe.

In addition, the combination of carbon dioxide and an alcohol is known to form alkoxylcarbonic acid, supposedly conferring some acidic character to SFC mobile phases. Direct measurements of the apparent pH are impossible, but colour indicators of pH can be used to define the range of apparent pH provided by carbon dioxide-alcohol mixtures, with or without additives. Five colour indicators (Thymol Blue, Bromocresol Green, Methyl Red, Bromocresol Purple, and Bromothymol Blue) were selected to provide a wide range of aqueous pKa values (from 1.7 to 8.9). UV–vis absorption spectra measured in liquid phases of controlled pH were compared to those measured with a diode-array detector employed in SFC, with the help of chemometric methods. Based on these observations, it is concluded that the apparent pH range in carbon dioxide-methanol mobile phases is close to 5. Increasing the proportion of methanol (in the course of a gradient elution for instance) causes decreasing apparent pH. Strong acids can further decrease the apparent pH below 1.7; strong bases have little influence on the apparent pH, probably because, in this range of concentrations, they are titrated by alkoxylcarbonic acid or form ion pairs with alkoxycarbonate. However, bases and salts could stabilize the acidity in the course of gradient runs.

https://doi.org/10.1016/j.chroma.2017.02.066

91. Chemometric Evaluation Of The Combined Effect Of Temperature, Pressure, And Co-Solvent Fractions On The Chiral Separation Of Basic Pharmaceuticals Using Actual Vs Set Operational Conditions

Abstract
Six pharmaceutical compounds were used as chiral model compounds. Combined effects of pressure, temperature, and modifier fraction were investigated. The fraction of modifier in eluent had the strongest effect on the retention. The use of set vs. actual conditions as input data in the DoE was evaluated. Significant differences were found between models using set vs. actual conditions.

The need to determine the actual operational conditions, instead of merely using the set operational conditions, was investigated for in packed supercritical fluid chromatography (SFC) by design of experiments (DoE) using a most important type of compounds, pharmaceutical basics, as models. The actual values of temperature, pressure, and methanol levels were recorded and calculated from external sensors, while the responses in the DoE were the retention factors and selectivity. A Kromasil CelluCoat column was used as the stationary phase, carbon dioxide containing varying methanol contents as the mobile phase, and the six racemates of alprenolol, atenolol, metoprolol, propranolol, clenbuterol, and mianserin were selected as model solutes. For the retention modeling, the most important term was the methanol fraction followed by the temperature and pressure. Significant differences (p < 0.05) between most of the coefficients in the retention models were observed when comparing models from set and actual conditions. The selectivity was much less affected by operational changes, and therefore was not severely affected by difference between set and actual conditions. The temperature differences were usually small, maximum ±1.4 °C, whereas the pressure differences were larger, typically approximately +10.5 bar. The set and actual fractions of methanol also differed, usually by ±0.4 percentage points. A cautious conclusion is that the primary reason for the discrepancy between the models is a mismatch between the set and actual methanol fractions. This mismatch is more serious in retention models at low methanol fractions. The study demonstrates that the actual conditions should almost always be preferred.
92. Systematic Investigations Of Peak Deformations Due To Co-Solvent Adsorption In Preparative Supercritical Fluid Chromatography

Keywords: Supercritical fluid chromatography; Co-solvent adsorption; Adsorption strength; Langmuir band shape; Anti-Langmuir band shape

Abstract
An adsorbing co-solvent can cause deformed elution profiles. This effect is general for several co-solvents/stationary phase combinations. A simple test can evaluate the risk of these deformations. All solute peak deformations due to co-solvents result from competition.

Strangely shaped overloaded bands were recently reported using a standard supercritical fluid chromatographic system comprising a diol column as the stationary phase and carbon dioxide with methanol as the mobile phase. Some of these overloaded elution profiles appeared strongly deformed and even had "anti-Langmuirian" shapes although their solute compounds had "Langmuirian" adsorption. To obtain a more complete understanding of the generality of these effects, the investigation was expanded to cover other also common co-solvents, such as ethanol, 2-propanol, and acetonitrile, as well as various stationary phase materials, such as silica, and 2-ethylpyridine. From this expanded study it could be confirmed that the effects of deformed overloaded solute band shapes, due to co-solvent adsorption, is general phenomena in supercritical fluid chromatographic. It could also be concluded that these effects as well as previously observed "solvent effects" or "plug effects" are entirely due to competition between the solute and solvent molecules for the adsorption sites on the stationary phase surface. Finally, guidelines were given for how to evaluate the risk of deformations occurring for a given solvent–column combination, based simply on testing retention times of solutes and co-solvent.

93. Enantioseparation Of Novel Chiral Sulfoxides On Chlorinated Polysaccharide Stationary Phases In Supercritical Fluid Chromatography

Keywords: Conformation; Enantiomer separation; Molecular modelling; Polysaccharide-based chiral stationary phase; Sulfoxides; Supercritical fluid chromatography

Abstract
Chlorinated polysaccharide-based stationary phases yield high retention and enantioseparation of sulfoxides
Enantiorecognition is more favorable for folded analyte conformations than for linear conformations
Stationary phase conformation changes significantly when methanol percentage reaches about 60%
Asymmetric sulfoxides is a particular case of chirality that may be found in natural as well as synthetic products. Twenty-four original molecules containing a sulfur atom as a centre of chirality were analyzed in supercritical fluid chromatography on seven polysaccharide-based chiral stationary phases (CSP) with carbon dioxide – methanol mobile phases. While all the tested CSP provided enantioseparation for a large part of the racemates, chlorinated cellulose phases proved to be both highly retentive and highly enantioselective towards these species. Favourable structural features were determined by careful comparison of the enantioseparation of the probe molecules. Molecular modelling studies indicate that U-shaped (folded) conformations were most favorable to achieve high enantioresolution on these CSP, while linear (extended) conformations were not so clearly discriminated.
For a subset of these species adopting different conformations, a broad range of mobile phase compositions, ranging from 20 to 100% methanol in carbon dioxide, were investigated. While retention decreased continuously in this range, enantioseparation varied in a non-monotonous fashion. Abrupt changes in the tendency curves of retention and selectivity were observed when methanol proportion reaches about 60%, suggesting that a change in the conformation of the analytes and/or chiral selector is occurring at this point.
94. Speed-Resolution Advantage Of Turbulent Supercritical Fluid Chromatography In Open Tubular Columns: II – Theoretical And Experimental Evidences

Keywords: Supercritical fluid chromatography; Laminar-to-turbulent flow regime; Open tubular columns; Golay equation; Kinetic plots; Reynolds number

Abstract

Golay's plate height equation extended from laminar to turbulent flow was used. Performance of open tubes in turbulent regime with carbon dioxide eluent is projected. Significant speed-resolution benefits are expected under turbulent flow regime. The theoretical predictions are in good agreement with experimental dispersion data.

The potential advantage of turbulent supercritical fluid chromatography (TSFC) in open tubular columns (OTC) was evaluated on both theoretical and practical viewpoints. First, the dispersion model derived by Golay in 1958 and recently extended from laminar to turbulent flow regime is used for the predictions of the speed-resolution performance in TSFC. The average dispersion coefficient of matter in the turbulent flow regime was taken from the available experimental data over a range of Reynolds number from 2000 to 6000. Kinetic plots are built at constant pressure drop ($\Delta P = 4500$ psi) and Schmidt number ($Sc = 15$) for four inner diameters (10, 30, 100, and 300 $\mu$m) of the OTC and for three retention factors (0, 1, and 10). Accordingly, in turbulent flow regime, for a Reynolds number of 4000 and a retention factor of 1 (the stationary film thickness is assumed to be negligible with respect to the OTC diameter), the theory projects that a 300 $\mu$m i.d. OTC has the same speed-resolution power (200,000 theoretical plates; 2.4 min hold-up time) as that of a 10 $\mu$m i.d. OTC operated in laminar flow regime.

Secondly, the experimental plate heights of $n$-butylbenzene are measured in laminar and turbulent flow regimes for a 180 $\mu$m $\times$ 4.8 m fused silica capillary column using pure carbon dioxide as the mobile phase. The back pressure regulator was set at 1500 psi, the temperature was uniform at 297 K, and the flow rate was increased step-wise from 0.50 to 3.60 mL/min so that the experimental Reynolds number increases from 700 to 5400. The experiments are in good agreement with the plate heights projected in TSFC at high flow rates and with those expected at low flow rates in a laminar flow regime.

95. Rapid Determination Of 9 Aromatic Amines In Mainstream Cigarette Smoke By Modified Dispersive Liquid Liquid Microextraction And Ultraperformance Convergence Chromatography Tandem Mass Spectrometry

Keywords: aromatic amines; dispersive liquid liquid microextraction; ultraperformance convergence chromatography tandem mass spectrometry; mainstream cigarette smoke

Abstract

A rapid and sensitive UPC$^2$-MS/MS method was developed and validated.

Nine aromatic amines were fully separated within 5.0 min.

Simplified sample preparation by modified DLLME

Organic solvents were dramatically reduced attributing to DLLME and UPC$^2$-MS/MS

The method was successfully applied to mainstream cigarette smoke.

Aromatic amines in mainstream cigarette smoke have long been monitored due to their carcinogenic toxicity. In this work, a reliable and rapid method was developed for the simultaneous determination of 9 aromatic amines in mainstream cigarette smoke by modified dispersive liquid microextraction (DLLME) and ultraperformance convergence chromatography tandem mass spectrometry (UPC$^2$-MS/MS). Briefly, the particulate phase of the cigarette smoke was captured by a Cambridge filter pad, and diluted hydrogen chloride aqueous solution is employed to extract the aromatic amines under mechanical shaking. After alkalization with sodium hydroxide solution, small amount of toluene was introduced to further extract and enrich aromatic amines by modified DLLME under vortexing. After centrifugation, toluene phase
was purified by a universal QuEChERS cleanup kit and was finally analyzed by UPC²-MS/MS.
Attributing to the superior performance of UPC²-MS/MS, this novel approach allowed the
separation and determination of 9 aromatic amines within 5.0 min with satisfactory resolution and
sensitivity. The proposed method was finally validated using Kentucky reference cigarette 3R4F,
and emission levels of targeted aromatic amines determined were comparable to previously
reported methods. At three different spiked levels, the recoveries of most analytes were ranged
from 74.01% to 120.50% with relative standard deviation (RSD) less than 12%, except that the
recovery of p-toluidine at low spiked level and 3-aminobiphenyl at medium spiked level was
62.77% and 69.37% respectively. Thus, this work provides a novel alternative method for the
simultaneous analysis of 9 aromatic amines in mainstream cigarette smoke.
https://doi.org/10.1016/j.chroma.2017.05.056

96. Simultaneous Quantification Of 11 Active Constituents In Shexiang Baoxin Pill
By Ultraperformance Convergence Chromatography Combined With Tandem
Mass Spectrometry
2017 - Journal of Chromatography B
Keywords: Shexiang Baoxin Pill; UPC2-MS/MS; Quantification; Non-volatiles; Volatiles
Abstract
Apply UPC²-MS/MS method to quantification of constituents in traditional Chinese medicines
(TCMs)
Simultaneous quantification of 11 components in SBP including volatiles and non-volatiles
Methods have advantages with its rapidity, sensitivity, short time and a wide range of analyzing
This work also detailed analyzed the effects of 11 components on 13 batches of SBP samples

On account of the complexity of chemical constituents of Shexiang Baoxin Pill (SBP), a famous
traditional Chinese medicine (TCM) formula, a novel and effective UPC²-MS/MS method was
developed to simultaneously determine the content of 11 active compounds of SBP with
outstanding separation ability. Eleven components in SBP, including 2 ginsenosides, 2 bile acids, 3
bufadienolides and 4 volatiles were detected by electrospray ionization tandem mass spectrometry
in positive and negative ion modes with multiple reaction monitor (MRM). The analysis was
performed at 30 °C using an Acquity UPC² Diol (3.0 × 50 mm, 1.7 μm) column with linear
gradient elution (eluent A, CO₂; eluent B, methanol containing 20 mM ammonium acetate), back
pressure of 2000 psi, flow rate of 1.2 mL/min and the injection volume of 1.0 μL. The method was
extensively validated regarding the linearity (r ≥ 0.9974), precision (≤3.11%), recovery (93.34–
104.50%), repeatability (≤2.00%) and stability (≤4.20%). Using this method, 11 active
compounds of SBP with different polarity were simultaneously quantified in one chromatography
analysis within 8 min. Statistical analysis of the effects of 11 compounds on the quality of SBP
revealed that the content of cinnamaldehyde varied widely in different batches. This work presents
an exemplary study for quality control of complex samples, especially for TCMs.
https://doi.org/10.1016/j.jchromb.2017.03.033

97. Effect Of Fatty Acid Chain Length On The Crystallization Behavior Of Trans-Free
Margarine Basestocks During Storage
2017 - Journal of Oleo Science
Keywords: fully hydrogenated Acer truncatum oil, fatty acids, trans-free, interesterification,
crystallization
Abstract
In order to obtain margarine free of trans-fatty acids, four interesterified basestocks were
prepared by chemical interesterification (CIE) of oil blends. Different ratios of palm stearin, palm
olein and soybean oil were mixed without and with 1) fully hydrogenated Acer truncatum oil
(FHATO), 2) fully hydrogenated rapeseed oil or 3) palm kernel oil containing a similar amount of
saturated, monounsaturated and polyunsaturated fatty acids, but different saturated fatty acid
length for CIE. Compared to the physical blends, the CIE samples demonstrated lower slip melting
points and decreased solid fat contents, especially at high temperatures, indicating that the CIE samples might have improved mouthfeel. In all CIE samples, the β crystal form disappeared and only the β’ crystal form was observed, except for sample 2, which contained a mixed β and β’ forms. Furthermore, in all CIE samples, except sample 1, the β’ crystal forms began transforming to β form after only two cycles of higher temperature treatments indicating that the CIE sample with FHATO had the most resistance to temperature fluctuation during storage which may be attributed to its longer saturated chains. In conclusion, the CIE basestocks containing longer saturated fatty acids could be more suitable for margarine use.

http://dx.doi.org/10.5650/jos.ess16210

98. Investigation Of The Effect Of Mobile Phase Composition On Selectivity Using A Solvent-Triangle Based Approach In Achiral SFC

2017 - Journal of Pharmaceutical and Biomedical Analysis

Keywords: Achiral SFC, Drug impurity profiling, Eluotropic strength, Separation optimization, Modifiers

Abstract:
Defining a method development methodology for achiral drug impurity profiling in SFC requires a number of steps. Initially, diverse stationary phases are characterized and a small number of orthogonal or dissimilar phases are selected for further method development. In this paper, we focus on a next step which is the investigation of the modifier composition on chromatographic selectivity. A solvent-triangle based approach is used in which blends of organic solvents, mainly ethanol (EtOH), propanol (PrOH), acetonitrile (ACN) and tetrahydrofuran (THF) mixed with methanol (MeOH) are tested as modifiers on six dissimilar stationary phases. The tested modifier blends were composed to have equal eluotropic strengths as calculated on bare silica. The modifier leads to minor changes in terms of elution order, retention and mixture resolution. However, varying only the modifier composition on a given stationary phase does not lead to the creation of dissimilar systems. Therefore the modifier composition is an optimization parameter, with the stationary phase being the factor determining most the selectivity of a given mixture in achiral SFC.

http://dx.doi.org/10.1016/j.jpba.2016.10.016

99. Quantitative Determination Of Salbutamol Sulfate Impurities Using Achiral Supercritical Fluid Chromatography

2017 - Journal of Pharmaceutical and Biomedical Analysis

Keywords: Supercritical fluid chromatography (SFC), Quality by design (QbD), Impurity profiling, Method validation, Total error approach, Salbutamol sulfate

Abstract:
In the last years, supercritical fluid chromatography has largely been acknowledged as a singular and performing technique in the field of separation sciences. Recent studies highlighted the interest of SFC for the quality control of pharmaceuticals, especially in the case of the determination of the active pharmaceutical ingredient (API). Nevertheless, quality control requires also the determination of impurities. The objectives of the present work were to (i) demonstrate the interest of SFC as a reference technique for the determination of impurities in salbutamol sulfate API and (ii) to propose an alternative to a reference HPLC method from the European Pharmacopeia (EP) involving ion-pairing reagent. Firstly, a screening was carried out to select the most adequate and selective stationary phase. Secondly, in the context of robust optimization strategy, the method was developed using design space methodology. The separation of salbutamol sulfate and related impurities was achieved in 7 min, which is seven times faster than the LC-UV method proposed by European Pharmacopeia (total run time of 50 min). Finally, full validation using accuracy profile approach was successfully achieved for the determination of impurities B, D, F and G in salbutamol sulfate raw material. The validated dosing range covered 50 to 150% of the targeted concentration (corresponding to 0.3% concentration level), LODs close to 0.5 μg/mL were estimated. The SFC method proposed in this study could be presented as a...
Fast Separation Of Flavonoids By Supercritical Fluid Chromatography Using A Column Packed With A Sub-2 μm Particle Stationary Phase

Keywords: flavonoids: dietary supplements; Radix astragali; Chinese medicine;

Abstract
The purpose of this study was to compare the effects of different chromatographic columns for the separation of seven flavonoids. Four different stationary phases are available, including bridged ethyl hybrid, BEH and the same hybrid phase modified with 2-ethylpyridine, CSH fluorophenyl, and HSS C18 SB. The analytes included calycosin, genistein, medicarpin, calycosin-7-O-β-d-glucoside, formononetin, formononetin-7-O-β-d-glucoside, and liquiritigenin. The CSH fluorophenyl column was determined to be the most suitable and provided the fastest separation within 17 min using gradient elution with carbon dioxide as the mobile phase and methanol as the co-solvent. Good peak shapes were obtained, and the values of the peak asymmetry were close to 1.0 for all of the flavonoids. The resolution was more than 1.41 for all of the separated peaks. Baseline separation on the optimal columns was achieved by changing the co-solvent type and adjusting the temperature and pressure. Quantitative performance was evaluated under optimized conditions, and method validation was accomplished. The validation parameters, such as linearity, sensitivity, precision, and accuracy, were satisfactory. Good repeatability of both peak area (relative standard deviation <1.02%) and retention time (relative standard deviation <0.88%) was observed. The optimized chromatographic methods were successfully used for the determination of seven flavonoids in Radix astragali. The sensitivity was sufficient for the analysis of real samples.

https://doi.org/10.1002/jssc.201601021
101. Ultra-Performance Convergence Chromatography For The Quantitative Determination Of Bioactive Compounds In *Aralia Continentalis* Kitagawa As Quality Control Markers

Keywords: Quality control, S.Korea, China

**Abstract**

A rapid ultra-performance chromatography method was developed for the quantitative determination of bioactive compounds in *Aralia continentalis* as quality control markers. Quantitative analysis indicated the presence of two major bioactive compounds: diterpenoid acids continentalic acid and kaurenoic acid. Using a Torus 1-aminoanthracene column, continentalic acid and kaurenoic acid were separated in less than 8 min. The method was validated with respect to precision, accuracy, and linearity according to the International Conference on Harmonization guidelines. The optimized method exhibited a good linear correlation \( r^2 > 0.996 \), excellent precision (RSD < 1.0%), and acceptable recoveries (99.97–100.26%). Limits of detection for continentalic acid and kaurenoic acid were 0.068 and 0.097 μg/mL, respectively, while their corresponding limits of quantitation were 0.207 and 0.295 μg/mL. The system performance of ultra-performance convergence chromatography was compared with that of conventional high-performance liquid chromatography with respect to analysis time and efficiency. The proposed method was found to be reliable and convenient for the quantitative analysis of continentalic acid and kaurenoic acid in *A. continentalis* from South Korea and *A. pubescens* from China. This study is expected to serve as a guideline for the quality control of *Aralia continentalis*. [https://doi.org/10.1002/jssc.201601261](https://doi.org/10.1002/jssc.201601261)

102. Simple, Rapid, And Environmentally Friendly Method For The Separation Of Isoflavones Using Ultra-High Performance Supercritical Fluid Chromatography

**Abstract**

Isoflavones are natural substances that exhibit hormone-like pharmacological activities. The separation of isoflavones remains an analytical challenge because of their similar structures. We show that ultra-high performance supercritical fluid chromatography can be an appropriate tool to achieve the fast separation of 12 common dietary isoflavones. Among the five tested columns the Torus DEA column was found to be the most effective column for the separation of these isoflavones. The impact of individual parameters on the retention time and separation factor was evaluated. These parameters were optimized to develop a simple, rapid and green method for the separation of the 12 target analytes. It only took 12.91 min using gradient elution with methanol as an organic modifier and formic acid as an additive. These isoflavones were determined with limit of quantitation ranging from 0.10 to 0.50 μg/mL, which was sufficient for reliable determination of various matrixes. [https://doi.org/10.1002/jssc.201601454](https://doi.org/10.1002/jssc.201601454)
103. Construction Of An Off-Line Two Dimensional Reversed-Phase Liquid Chromatography /Ultra-High Performance Supercritical Fluid Chromatography Method For Rapid And Comprehensive Analysis Of Piper Kadsura

Keywords: Ultra-high performance supercritical fluid chromatography; Reversed-phase liquid chromatography; Two-dimensional chromatography; Rapid analysis; Piper kadsura

Abstract
A 2D-RPLC/UHPSFC method for rapid and comprehensive analysis of Piper kadsura. UHPSFC analysis of all fractions prepared from RPLC was within 3.0 min. 1033 peaks were identified using the 2D-RPLC × UHPSFC with excellent orthogonality. In this study, ultra-high performance supercritical fluid chromatography (UHPSFC) was chosen to construct an off-line two dimensional chromatography with reversed-phase liquid chromatography (RPLC) for rapid and comprehensive analysis of Piper kadsura. The sample was firstly divided into a petroleum ether (PE) extract and an ethyl acetate (EA) extract, and then they were separated into 21 and 28 fractions in the first dimensional RPLC, respectively. In the second dimension, the effect of UHPSFC parameters on the analysis speed was investigated. Each PE fraction could be analyzed in 2.0 min, and every EA fraction could be analyzed in 3.0 min. In total, at least 1033 peaks were detected by this method, including 204 peaks from the PE fractions and 829 peaks from the EA fractions. The 2D-RPLC/UHPSFC system with the characteristics of fast separation and high orthogonality has a great potential for the rapid and comprehensive analysis of the complex samples.

https://doi.org/10.1016/j.supflu.2017.03.004

104. Fast And Green – CO₂ Based Extraction, Isolation, And Quantification Of Phenolic Styrax Constituents

Keywords: Liquidambar orientalis - Hamamelidaceae - supercritical fluid extraction - supercritical fluid chromatography - ultra-performance convergence chromatography – styrax

Abstract
In this study the first supercritical fluid based protocol for the extraction, analysis, and isolation of six polar compounds, i.e., o-vanillin (1), styracin (2), vanillin (3), trans-cinnamic acid (4), vanillic acid (5), and shikimic acid (6), was developed. First, eight styrax resin products (R1–R8) obtained from various Liquidambar tree species, which are known to contain compounds 2–6, were extracted with a 1:1 mixture of supercritical CO₂ and EtOH. Within 4 minutes, the compounds were successfully baseline separated on an Acquity UPC² BEH 2-EP (3.0 × 100 mm, 1.7 μm) column using a mobile phase of supercritical CO₂ and MeOH with 0.1% phosphoric acid. The compounds were quantified and the method was validated according to current ICH guidelines.
Scaling up to preparative supercritical fluid chromatography using a Viridis BEH 2-EP (10 × 250 mm, 5 µm) column allowed for a fast separation and isolation of the selected constituents 2 and 4 from R6 within 7 minutes. This supercritical fluid protocol is easily adaptable to compounds of similar polarity. The increase in speed and its environmental friendliness underline its superiority over conventional set-ups.

http://dx.doi.org/10.1055/s-0043-105499

105. Development And Application Of Sub-2-Mm Particle CO₂-Based Chromatography Coupled To Mass Spectrometry For Comprehensive Analysis Of Lipids In Cottonseed Extracts

2017 - Rapid Communications in Mass Spectrometry

Keywords: Cotton Seed Oil, food and chemical industries; triacylglycerols; biological lipid extracts; lipid profiling

Abstract
Refined cottonseed oil has widespread applications in the food and chemical industries. Although the major lipids comprising cottonseed oil (triacylglycerols) are well known, there are many diverse lipid species in cotton seeds that occur at much lower levels and have important nutritional or anti-nutritional properties.

The lipid technical samples were prepared in chloroform. The biological samples were extracted using a mixture of isopropanol/chloroform/H₂O (2:1:0.45). The data were collected using high and low collision energy with simultaneous data collection on a time-of-flight (TOF) mass spectrometer which allowed the characterization of lipids by precursor and product ion alignment. The supercritical fluid chromatography methodology is flexible and can be altered to provide greater retention and separation. The comprehensive method was used to screen seed lipid extracts from several cotton genotypes using multivariate statistical analysis.

Method variables influencing the peak integrity and chromatographic separation for a mixture of lipids with different degrees of polarity were explored. The experiments were designed to understand the chromatographic behavior of lipids in a controlled setting using a variety of lipid extracts. Influences of acyl chain length and numbers of double bonds were investigated using single moiety standards.

The methodology parameters were examined using single moiety lipid standards and standard mixtures. The method conditions were applied to biological lipid extracts, and adjustments were investigated to manipulate the chromatography. Insights from these method variable manipulations will help to frame the development of targeted lipid profiling and screening protocols.


106. Enantioselective 4-Hydroxylation Of Phenols Under Chiral Organoiodine(I/III) Catalysis

2017 - Synthesis

Keywords: catalysis, chiral, dearomatization, hydroxylation, hypervalent iodine, oxidation

Abstract
A procedure for the intermolecular enantioselective dearomatization of phenols under chiral (I/III) catalysis is reported. This protocol employs 3-chloroperoxybenzoic acid (m-CPBA) as the terminal oxidant together with a defined C₂-symmetric aryl iodide as the effective organocatalyst. This enantioselective reaction proceeds with complete selectivity under mild conditions and enables the hydroxylative dearomatization of a number of phenols to give the corresponding p-quinol products with up to 50% ee.

HPLC measurements were carried out on a Acquity UPLC® system (Waters) UltraPerformance Convergence Chromatography™ (UPC²™ ) equipped with a detector Acquity UPLC PDA (Photodiode Array detector). The respective chiral stationary phase (Chiralpak IA, IC or IE) and exact conditions are specified for each individual compound within the compound characterization section.

http://dx.doi.org/10.1055/s-0036-1588808
107. Coupling Of Supercritical Fluid Chromatography To Mass Spectrometry For The Analysis Of Dechlorane Plus: Examination Of Relevant Negative Ion Atmospheric Pressure Chemical Ionization Mechanisms

Keywords: Supercritical fluid chromatography; Negative APCI/MS; Dechlorane Plus; Ionization; Superoxide; Environmental analysis

Abstract
The analysis of Dechlorane Plus (DP) by pSFC-APCI/MS is investigated. APCI pathways are discussed and triethylamine is optimized as an APCI reagent.
Superoxide formation is correlated to APCI probe temperature and DP analyte response

During an investigation of the potential associated with coupling packed column supercritical fluid chromatography (pSFC) to mass spectrometry for the analysis of Dechlorane Plus and related compounds, it was found that negative ion atmospheric pressure chemical ionization (APCI) was a promising ionization technique. In the course of maximizing the responses associated with the target analytes, it proved useful to examine some aspects of the complex nature and reactivity of the corona discharge plasma generated to explain the observed ionization products. Various dopants/reagents were screened for both APCI and atmospheric pressure photoionization (APPI) in negative ion mode and mechanisms of ionization involving superoxide were elucidated based on the results obtained. Superoxide formation was found to be temperature dependent and directly related to the intensity of the ion cluster \([M-\text{Cl}+\text{O}]^-\) obtained for the target DP analytes. Furthermore, triethylamine was identified as a reagent capable of suppressing unwanted side reactions during the ionization process and maximizing response associated with the analytes of interest. The applicability of pSFC-APCI/MS for the separation and detection of Dechlorane Plus and related compounds was demonstrated by analyzing Lake Ontario sediment and comparing the results with values reported in the scientific literature.

https://doi.org/10.1016/j.talanta.2017.04.066

108. Advanced Analytical Techniques For Fat-Soluble Vitamin Analysis

Keywords: Fat soluble vitamins; HPLC; UHPLC; Supercritical fluid chromatography; Convergence chromatography; Miniaturized chromatographic techniques; Core-shell technology; Sample preparation; Miniaturized sample preparation techniques; Vitamin profiling

Abstract

This review presents advancements in sample preparation and liquid chromatographic methods made during the last 10 years for the fat-soluble vitamin (FSV) analysis in foods. Since activity and bioavailability of organic micronutrients (MOs) depend on the form in which they are present in a food, special emphasis has been given to the most recent techniques, instruments and approaches which have allowed the extraction and separation of various vitamin homologues as well as the comprehensive MO profiling in foods. Due to the numerous ancillary advantages, miniaturized techniques which have been applied in this analysis field are also discussed in this review. A number of selected applications are proposed to enable readers to access the most recent developments and trends aimed at gaining more in-depth knowledge of the vitamin composition of foods.

http://dx.doi.org/10.1016/j.trac.2016.12.001

109. Effects Of Free Fatty Acids On Insulin And Glucagon Secretion

2017 - PhD Thesis submission Uppsala University
Free fatty acid analysis A sample of 100 µl plasma was vortexed thoroughly with a 60 µl mixture of internal standards (3.33 µg/ml of nonanoic acid (C9:0) 3.33 mg/ml, 16.66 µg/ml of heptanoic acid (C17:0), and 3.33 µg/ml of tricosylic acid (C23:0)). Plasma lipids were extracted with 2 ml of organic solvent mixture (isopropanol: heptane: 1 M hydrochloric acid (40:10:1, v/v/v)) by vortexing for 30 min. During the extraction, lipids need to be protected against oxidation by adding 0.05 mg/ml butylated hydroxytoluene to the organic solvent mixture. The samples were incubated at room temperature for 10 min. One ml of water was added to the samples and the plasma lipids were extracted into 2 ml of heptane. Another lipid extraction step was performed by adding 1 ml of heptane. The heptane phase was dried under nitrogen gas and the dried lipids were redissolved in 100 µl of heptane. The sample was stored at -20°C until Ultra-Performance Convergence Chromatography (UPC2) analysis. FFAs were analyzed with UPC2 (Waters ACQUITY® UPC2™, Milford, MA) coupled with tandem MS (XEVO® TQ-S, Milford, MA). The analysis was performed with an Acquity UPC2 HSS C18 column (100 mm 3.0 mm, 1.8 µm at 40 °C, Waters, Milford, MA, USA). The mobile phase A is 99.99% pure compressed CO2. Methanol containing 0.1% formic acid was used as the co-solvent.

110. Quantification Of The Neurotransmitters Melatonin And N-Acetyl-Serotonin In Human Serum By Supercritical Fluid Chromatography Coupled With Tandem Mass Spectrometry

Keywords: Melatonin, N-acetyl-serotonin, UHPLC-MS/MS, SFC-MS/MS

Abstract

The aim of this study was developing a supercritical fluid chromatography tandem mass spectrometry (SFC-MS/MS) method and an ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method, for the analysis of N-acetyl-serotonin (NAS) and melatonin (Mel) in human serum, and to compare the performance of these methods. Deuterated isotopologues of the neurotransmitters were synthesized and evaluated for suitability as internal standards in sample preparation. Liquid-liquid extraction was selected as sample preparation procedure. With chloroform, the best extraction solvent tested, an extraction yield of 48 ± 2% for N-acetyl-serotonin and 101 ± 10% for melatonin was achieved. SFC separation was accomplished within 3 min on a BEH stationary phase, employing isocratic elution with 90% carbon dioxide and 0.1% formic acid as well as 0.05% ammonium formate in methanol. For the 4 min UHPLC gradient separation with 0.1% formic acid in water and methanol, respectively, a Kinetex XB-C18 was used as stationary phase. Both chromatographic techniques were optimized regarding mobile phase composition, additives to the mobile phase and column temperature. Multiple reaction monitoring (MRM) analysis was used for quantification of the metabolites. Both methods were validated regarding retention time stability, LOD, LOQ, repeatability and reproducibility of quantification, process efficiency, extraction recovery and matrix effects. LOD and LOQ were 0.017 and 0.05 pg μL⁻¹ for NAS and 0.006 and 0.018 pg μL⁻¹ for Mel in SFC-MS/MS compared to 0.028 and 0.1 pg μL⁻¹ for NAS and 0.006 and 0.017 pg μL⁻¹ for Mel in UHPLC-MS/MS.

http://dx.doi.org/10.1016/j.aca.2016.08.012

111. A Fast And Sensitive Method For The Separation Of Carotenoids Using Ultra-High Performance Supercritical Fluid Chromatography Mass Spectrometry

Keywords: Lund University, Carotene, Xanthophyll, Orthogonal column screening, Method development, Supercritical fluid, Mass spectrometry

Abstract

In this study, a rapid and sensitive ultra-high performance supercritical fluid chromatography-mass spectrometry (UHPSFC-MS) method has been developed and partially validated for the
separation of carotenoids within less than 6 min. Six columns of orthogonal selectivity were examined, and the best separation was obtained by using a 1-aminoanthracene (1-AA) column. The length of polyene chain as well as the number of hydroxyl groups in the structure of the studied carotenoids determines their differences in the physiochemical properties and thus the separation that is achieved on this column. All of the investigated carotenoids were baseline separated with resolution values greater than 1.5. The effects of gradient program, back pressure, and column temperature were studied with respect to chromatographic properties such as retention and selectivity. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) were compared in both positive and negative mode, using both direct infusion and hyphenated with UHPSFC. The ESI in positive mode provided the highest response. The coefficient of determination ($R^2$) for all calibration curves were greater than 0.998. Limit of detection (LOD) was in the range of 2.6 and 25.2 ng/mL for $\alpha$-carotene and astaxanthin, respectively, whereas limit of quantification (LOQ) was in the range of 7.8 and 58.0 ng/mL for $\alpha$-carotene and astaxanthin, respectively. Repeatability and intermediate precision of the developed UHPSFC-MS method were determined and found to be RSD < 3 % and RSD < 6 %, respectively. The method was applied in order to determine carotenoids in supercritical fluid extracts of microalgae and rosehip.

http://dx.doi.org/10.1007/s00216-016-9707-5

112. Regioisomeric And Enantiomeric Analysis Of Triacylglycerols

Keywords: Institute of Microbiology, University of Chemistry and Technology, regioisomers, enantiomers, triacylglycerols, health science

Abstract

A survey of useful methods for separation and identification of regioisomers and enantiomers of triacylglycerols. Gas chromatography, gas chromatography-mass spectrometry, $^{13}$C NMR determination of regioisomers by enzymatic methods, and supercritical fluid chromatography are briefly surveyed, whereas a detailed description is given of the analysis of triacylglycerols by liquid chromatography, especially with silver ion (Ag$^+$; argentation), and nonaqueous reversed phase liquid chromatography. Special attention is paid to chiral chromatography. Details of mass spectrometry of triacylglycerols are also described, especially the identification of important triacylglycerol ions such as [M + H-RCOOH]$^+$ in atmospheric pressure chemical ionization mass spectra.

http://dx.doi.org/10.1016/j.ab.2016.05.028

113. Enantioselective Separation Of Biologically Active Basic Compounds In Ultra-Performance Supercritical Fluid Chromatography

Keywords: Charles University, University of Graz, chiral compounds, enantioselectivity, Amylose-based chiral stationary phase, health science

Abstract

http://dx.doi.org/10.1016/j.aca.2016.08.005
The enantioseparation of basic compounds represent a challenging task in modern SFC. Therefore this work is focused on development and optimization of fast SFC methods suitable for enantioseparation of 27 biologically active basic compounds of various structures. The influences of the co-solvent type as well as different mobile phase additives on retention, enantioselectivity and enantioresolution were investigated. Obtained results confirmed that the mobile phase additives, especially bases (or the mixture of base and acid), improve peak shape and enhance enantioresolution. The best results were achieved with isopropylamine or the mixture of isopropylamine and trifluoroacetic acid as additives. In addition, the effect of temperature and back pressure were evaluated to optimize the enantioseparation process. The immobilized amylose-based chiral stationary phase, i.e. tris(3,5-dimethylphenylcarbamate) derivative of amylose proved to be useful tool for the enantioseparation of a broad spectrum of chiral bases.

The chromatographic conditions that yielded baseline enantioseparations of all tested compounds were discovered. The presented work can serve as a guide for simplifying the method development for enantioseparation of basic racemates in SFC.

http://dx.doi.org/10.1016/j.aca.2016.04.044

114. Is The Solvation Parameter Model Or Its Adaptations Adequate To Account For Ionic Interactions When Characterizing Stationary Phases For Drug Impurity Profiling With Supercritical Fluid Chromatography?

2016 - Analytica Chimica Acta

Keywords: Vrije Universiteit, Université d’Orléans, solvation parameters, pharmaceuticals

Abstract

Nine commercially available polar and aromatic stationary phases were characterized under supercritical fluid chromatographic (SFC) conditions. Retention data of 64 pharmaceutical compounds was acquired to generate models based on the linear solvation energy relationship (LSER) approach. Previously, adaptation of the LSER model was done in liquid chromatography by the addition of two solute descriptors to describe the influence of positive (D⁺) and negative (D⁻) charges on the retention of ionized compounds. In this study, the LSER models, with and without the ionization terms for acidic and basic solutes, were compared. The improved fits obtained for the modified models support inclusion of the D⁺ and D⁻ terms for pharmaceutical compounds. Moreover, the statistical significance of the new terms in the models indicates the importance of ionic interactions in the retention of pharmaceutical compounds in SFC. However, unlike characterization through the retention profiles, characterization of the stationary phases by modelling never explains the retention variance completely and thus seems less appropriate.

http://dx.doi.org/10.1016/j.aca.2016.04.014

115. Simultaneous Determination Of Topiramate, Carbamazepine, Oxcarbazepine And Its Major Metabolite In Human Plasma By SFC-ESI-MS/MS With Polarity Switching: Application To Therapeutic Drug Monitoring

2016 - Arabian Journal of Chemistry

Keywords: Antiepileptic drugs, SFC-ESI-MS/MS, Human plasma, Therapeutic drug monitoring

Abstract
Antiepileptic drugs are the first choice for epilepsy treatment. Monitoring antiepileptic drugs is important to minimize their adverse side effects by choosing the optimum drug dosage. An accurate and high throughput supercritical fluid chromatography-tandem mass spectrometry method has been developed for the simultaneous quantification of several antiepileptic drugs in human plasma. Plasma samples were extracted with ethyl acetate and the upper organic layer was directly injected into the supercritical fluid chromatography/mass spectrometry (SFC-MS/MS) system without further nitrogen evaporation and subsequent reconstitution. The analytes were eluted on a UPCTM BEH, 2-EP column (100 × 3 mm, 1.7 μm) at a flow rate of 1.0 mL/min and multi-reaction monitoring (MRM) was performed for determination of the analytes and internal standard (IS) in polarity switching mode. Calibration curves were linear over the concentration ranges of 0.08–40, 0.01–15, 0.01–8 and 0.5–50 μg/mL with lower limit of quantifications of 0.08, 0.01, 0.01 and 0.50 μg/mL for topiramate, carbamazepine, oxcarbazepine and monohydroxycarbamazepine, respectively. This sensitive, accurate, novel method will be very useful for monitoring the above antiepileptic drugs and for pharmacokinetic studies.

http://dx.doi.org/10.1016/j.arabjc.2016.09.016

116. Ultraperformance Convergence Chromatography-High Resolution Tandem Mass Spectrometry For Lipid Biomarker Profiling And Identification

Keywords: Lipids, biological samples, mouse lung tissue, intra class species, tandem mass spec, ceramides; convergence chromatography, lipidomics, lung irradiation, supercritical fluid chromatography

Abstract

Lipids represent biologically ubiquitous and highly dynamic molecules in terms of abundance and structural diversity. Whereas the potential for lipids to inform on disease/injury is promising, their unique characteristics make detection and identification of lipids from biological samples analytically demanding. We report the use of ultraperformance convergence chromatography (UPC²), a variant of supercritical fluid chromatography, coupled to high-resolution, data-independent tandem mass spectrometry for characterization of total lipid extracts from mouse lung tissue. The UPC² platform resulted in lipid class separation and when combined with orthogonal column chemistries yielded chromatographic separation of intra-class species based on acyl chain hydrophobicity. Moreover, the combined approach of using UPC² with orthogonal column chemistries, accurate mass measurements, time-aligned low- and high-collision energy total ion chromatograms, and positive and negative ion mode product ion spectra correlation allowed for confident lipid identification. Of great interest was the identification of differentially expressed ceramides that were elevated 24 h post whole thorax lung irradiation. The identification of lipids that were elevated 24 h post-irradiation signifies a unique opportunity to investigate early mechanisms of action prior to the onset of clinical symptoms in the whole thorax lung irradiation mouse model.

http://dx.doi.org/10.1002/bmc.3822

117. Synthesis Of Novel Amphiphilic Hyaluronan Containing-Aromatic Fatty Acids For Fabrication Of Polymeric Micelles

Keywords: Contipro a.s., Tomáš Baťa University in Zlín, University Pardubice, hyaluronic acid, ω-Phenylalkanoic acids, amphiphilic polysaccharides, resveratrol, retinyl palmitate, hyaluronan, benzoyl chloride, 4-Phenylbutanoic acid, separation science

Abstract

Novel hydrophobized hyaluronan (HA) derivatives, containing ω-phenylalkanoic acids (ω-PAA, 4-phenylbutyric acid, 6-phenylhexanoic, 8-phenyloctanoic or 11-tolylundecanoic acids) were prepared by esterification. Mixed anhydrides obtained after reaction of the carboxyl acid moiety and benzoyl chloride were found to be active acylating agents, affording hydrophobized HA in good yield and under mild conditions. The reactivity of the aromatic fatty acids towards esterification has decreased with the increasing length of the aliphatic spacer between the aromatic substituent and carboxylic acid moiety. The novel HA derivatives self-assembled from very low concentrations.
and were found to be non-cytotoxic. The potential use of ω-phenylalkanoic acids grafted-HA towards drug delivery applications was demonstrated by hydrophobic drugs (resveratrol and retinyl palmitate) encapsulation. The drug loading capacity of the novel HA derivatives was significantly improved most likely because of n⋯n interactions between the micelle core and loaded hydrophobic aromatic compound.

doi:10.1016/j.carbpol.2016.06.085

118. Determination Of Stereoisomers In Landiolol Hydrochloride By Ultra Performance Convergence Chromatography

2016 - Chinese Journal of Analytical Chemistry

Keywords: Ultra performance convergence chromatography; Landiolol hydrochloride; Stereoisomers; Chiral separation

Abstract
A new method for chiral separation and purity inspection of landiolol hydrochloride and its stereoisomers was developed by ultra-performance convergence chromatography (UPC²). The mobile phase was the mixture of supercritical CO₂ and methanol/n-butyl alcohol/acetonitrile (1:1:1, V/V) plus 0.5% NH₃·H₂O. The separation was carried out on the Daicel CHIRALPAK®IF column (150 mm×4.6 mm, 3 μm) with a flow rate of 2.8 mL/min at 50°C using 223 nm as detection wavelength. Under the optimized experimental conditions, for R,R-stereoisomer, R,S-stereoisomer and S,R-stereoisomer, the detection limits (LOD, S/N=3) were 0.3, 0.4 and 0.3 mg/L, the linear ranges were 2-300 mg/L, 5-300 mg/L and 2-300 mg/L, the recoveries of spike samples were 103.4%±2.5%, 91.8%±2.5% and 101.7%±1.5%, and the injection repeatabilities were 0.06%, 0.09% and 0.08% (n=6), respectively. The experimental results demonstrate that the UPC²-based method can be used for the analysis and determination of landiolol hydrochloride and its stereoisomers.


119. Residue Determination Of Cis-Epoxiconazole Enantiomers In Fruit And Tea By Ultra Performance Convergence Chromatography Combined With Quardrupole Time-Of-Flight Mass Spectrometry

2016 - Chinese Journal of Analytical Chemistry

Keywords: Chiral enantiomers; Residue analysis; cis-Epoxiconazole; Apple; Grape; Tea; Ultra performance convergence chromatography; QTof mass spectrometry

Abstract
A chiral separation and residue determination method for cis-epoxiconazole enantiomers in apple, grape and tea samples was developed and validated by ultra performance convergence chromatography combined with quadrupole time-of-flight mass spectrometry (UPC²-QTOF/MS). The Chiral CCA column was used to separate cis-epoxiconazole enantiomers and the chromatography conditions (mobile phase modifier and proportion, column temperature, automated backpressure regulator, and auxiliary solvent) were optimized. Samples were extracted by acetonitrile, and respectively purified by Cleanert TPT or Pesti-Carb solid phase extraction (SPE) columns, then analyzed by UPC²-QTOF/MS. The optimum conditions were as follows: mobile phase was CO₂/isopropanol(95:5, V/V), flow-rate was 2.0 mL/min, automated backpressure regulator (ABPR) was 13.79 MPa, column temperature was 30°C, with a post-column auxiliary solvent of methanol/water (1:1, V/V) containing 2 mmol/L ammonium formate. The analyte was quantified by matrix external standard method. The results showed that linear range of this method was 0.01-1.00 mg/L, and the correlation coefficients were above 0.99. The recoveries of cis-epoxiconazole enantiomers at three spiked levels (0.005, 0.025 and 0.25 mg/kg) in fruit matrix were 67.9%-92.8% with relative standard deviations (RSDs, n=6) less than 10%, and the limit of quantification (LOQ) of enantiomers was 0.005 mg/kg. The recoveries of cis-epoxiconazole enantiomers at three spiked levels (0.01, 0.05 and 0.5 mg/kg) in black tea were 74.1%-84.0% with RSDs (n=6) less than 8%, and the LOQ for these two enantiomers was 0.01 mg/kg. This method is rapid, convenient and reliable, and could meet the requirement of residue analysis.


120. Separation Of Pharmaceuticals By SFC Using Mono And Di-Hydroxy Substituted Phenyl Stationary Phases
Keywords: Cannabinoids, chiral, synthetic

Abstract
Ultra high performance supercritical fluid chromatography (UHPSFC) technology presents an alternative and orthogonal solution to GC and HPLC for the separation of synthetic cannabinoids. In particular, it offers excellent selectivity for structural analogs and stereoisomers. Here, a simple method development strategy is demonstrated for the chiral analysis of selected synthetic cannabinoids including: HU-210, HU-211, (±)-CP 47,497, (±)-epi CP 47,497, (±)-CP 55,940 and (±)-epi CP 55,940. Fast stereoisomeric separations, including related enantiomers and diastereomers, are achieved on the Waters ACQUITY UPC2 System using the Waters Trefoil chiral columns. The efficiency of UHPSFC is demonstrated for rapid enantiomeric separation of chiral synthetic cannabinoids. - See more at: http://www.chromatographytoday.com/articles/supercritical-fluid-sfcgreen-chromatography/45/by_jacquelyn_runco_andrew_aubin_jo-ann_jablonski/simple_method_development_for_the_separation_of_chiral_synthetic_cannabinoids_using_ultra_high_performance_supercritical_fluid_chromatography/2090/#sthash.Zg7dEbKS.dpuf

121.Chiral Bioaccumulation Behavior Of Tebuconazole In The Zebrafish (Danio Rerio)

Keywords: Shenyang Agricultural University, Chinese Academy of Agricultural Sciences, State Key Laboratory for Biology of Plant Diseases and Insect Pests, tebuconazole, zebrafish, bioaccumulation, health science

Abstract
Tebuconazole is an effective chiral fungicide, and previous studies have demonstrated that tebuconazole enantiomers exhibit enantioselective toxicity to non-target aquatic organisms. Thus, the aim of the present study was to investigate the chiral bioaccumulation behavior of tebuconazole in zebrafish (Danio rerio). Two exposure concentrations (0.107 and 1.07 mg/L) of tebuconazole were used. The uptake experiments lasted for 8 days, and subsequently, the zebrafish were transferred to another clean tank containing water without tebuconazole for depuration experiments (up to 14 days). A significant trend in enantioselective bioaccumulation was observed in these zebrafish with the preferential accumulation of (−)-R-tebuconazole at two dose levels. The results of the depuration experiments indicated that the degradation of (−)-R-tebuconazole in zebrafish was slower than that of (+)-S-tebuconazole. The BCFk values for (+)-S-tebuconazole and (−)-R-tebuconazole in a low dose of this chemical were 11.22 and 16.25, respectively, while at a high dose, these values were 9.79 and 10.31, respectively. The enantiomer fraction of tebuconazole in zebrafish and water ranged from 0.31-0.49. Hence, future research should focus on the fate of tebuconazole in the aquatic environment at the enantiomer levels.
http://dx.doi.org/10.1016/j.ecoenv.2015.12.007

122.Fat-Soluble Vitamin And Carotenoid Analysis In Cooking Oils By Ultra-Performance Convergence Chromatography

Keywords: Fat soluble vitamins (FSV), Carotenoids, Ultra performance convergence chromatography (UPC2), Cooking oils

Abstract
In the current study, a rapid ultra-performance convergence chromatography (UPC²) method for the determination of seven fat soluble vitamins (vitamin A: retinol, retinyl acetate; vitamin D: ergocalciferol, cholecalciferol; vitamin E: α-tocopherol; vitamin K: phylloquinone, menaquinone) and three carotenoids (lutein, lycopene, β-carotene) in various cooking oils was developed. Fat soluble vitamins could be separated within 8 min on a UPC² system with a BEH column (3.0 × 100 mm, 1.7 μm) at 50 °C, using a gradient elution with a mobile phase of carbon dioxide and 2-propanol (99.9:0.1/99.2:0.8/99.9:0.1), at a flow rate of 2 mL/min and the automatic back pressure regulator (ABPR) set to 1800 psi. Carotenoids were separated within 3 min on a similar UPC² system with an HSS C18 SB column (3.0 × 100 mm, 1.8 μm) at 40 °C, under isocratic conditions with a mobile phase of carbon dioxide and ethanol (75:25; v/v). The flow rate was set
to 1.5 mL/min and the ABPR to 1800 psi. The limits of detection (LODs) and quantification (LOQs) were ranging between 0.01 and 1.17 μg/mL and from 0.05 and 3.59 μg/mL for fat soluble vitamins while carotenoid detection limits fell in the range of 0.03–0.11 μg/mL and of 0.10–0.38 μg/mL, respectively. The results showed excellent linearity for both methods ($R^2$ 0.9993–0.9999). A recovery study with standard addition technique into a selected coconut oil sample resulted in more than 90 % recovery for retinyl acetate, retinol, and vitamin K$_1$ and K$_2$, whereas the recovery for vitamin D$_2$ and D$_3$ ranged between 70 and 80 %. The lowest recovery of 68–70 % was found for α-tocopherol while almost comparable recovery rates above 80 % were observed for all three carotenoids.

http://dx.doi.org/10.1007/s12161-016-0661-9

123. Simultaneous Analysis Of Eight Vitamin E Isomers In Moringa Oleifera Lam. Leaves By Ultra Performance Convergence Chromatography

Keywords: Chinese Academy of Tropical Agricultural Sciences Center for Food Quality Supervision and Testing Ministry of Agriculture (Zhanjiang), Agricultural Product Processing Research Institute, Chinese Academy of Tropical Agricultural Sciences, α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol, food

Abstract

A new method for simultaneous determination of eight vitamin E isomers including α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol by ultra-performance convergence chromatography (UPC$^2$) equipped with a diode array detector was reported. They were separated on a BEH 2-EP column (3.0 mm × 100 mm, 1.7 μm) using gradient elution (95:5–80:20) with a mobile phase consisted of CO$_2$ and methanol:isopropanol (1:1, v/v), back pressure of 1800 psi, flow rate of 1.5 ml/min and detection at 294 nm. The results showed good linearity ($R^2$ = 0.9990–0.9998) and high resolution (1.48–7.67). Limits of detection (LOD) and quantification (LOQ) ranged from 23–49 ng/L and 70–150 ng/L, respectively. Relative standard deviations (RSD) for repeatability and reproducibility were 0.62–3.16% and 0.82–3.34%, respectively. Moreover, this method was successfully applied to analysis the vitamin E isomers in Moringa oleifera leaf samples.

http://dx.doi:10.1016/j.foodchem.2016.03.089

124. Vitamin E Analysis By Ultraperformance Convergence Chromatography And Structural Elucidation Of Novel (Alpha)Tocodienol By High Resolution Mass Spectrometry

Keywords: Palm Nutraceuticals, Research Instruments SDN, Matrix Analytical Technologies, tocodienol, palm oil, α-Tocodienol, tocopherol, tocotrienol, vitamins, food

Abstract

We have developed a method for analysing vitamin E using ultra-performance convergence chromatography with a chromatographic runtime of 5.5 min. A well-resolved chromatogram with excellent precision in retention time revealed seven vitamin E components in the palm oil derived tocotrienol-rich fraction. The major vitamin E components were α-tocopherol, α-tocotrienol, γ-tocotrienol and δ-tocotrienol whereas the minor vitamin E components were α-tocomonoenol, β-tocotrienol and an unreported trace component. The new component was positively identified by high-resolution mass spectrometry as 2-methyl-2(4′,8′,12′-trimethyltrideca-7′,11′-dienyl)5,7,8-trimethylchroman-6-ol or α-tocodienol

http://dx.doi.org/10.1016/j.foodchem.2015.09.073

125. A Comparative Study Of Triacylglycerol Composition In Chinese Human Milk Within Different Lactation Stages And Imported Infant Formula By SFC Coupled With Q-TOF-MS

Keywords: Human milk, Infant formula, Triacylglycerol, Supercritical fluid chromatography

Abstract

Triacylglycerols (TAGs) as the major component of milk fat are significant factors to ensure the healthy growth of infants. An efficient method for identifying TAGs in human milk (HM) and infant
formula (IF) was established using supercritical fluid chromatograph (SFC) coupled with quadruple time-of-flight mass spectrometry (Q-TOF-MS). The results indicated the feasibility of this method with satisfactory recoveries (>80%) and correlation coefficients ($r^2 > 0.993$). More than 60 TAGs in HM and 50 TAGs in IF were identified. The profiling results demonstrated that TAGs in HM were greatly affected by lactation stage. Significant differences were found between HM and IF, such as much higher medium chain TAGs and saturated TAGs in IF, indicating that the formulas developed by foreign manufacturers were not suitable for Chinese babies. This high-throughput method exhibits a huge potential for analysis of milk samples and the result may serve as an important guide for Chinese infants diet.

http://dx.doi.org/10.1016/j.foodchem.2016.09.099

126. Tocopherols And Tocotrienols In Plants And Their Products: A Review On Methods Of Extraction, Chromatographic Separation, And Detection
2016 - Food Research International

Keywords: Konkuk University, tocols, antioxidants, food, tocochromanols, vitamin E, fruits, vegetables, grains, food

Abstract
Vitamin E consists of four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ-, and δ-tocotrienols), collectively referred to as tocochromanols or tocols. Tocols are well-known for potent antioxidant, anticancer, anti-inflammatory, immuno-stimulatory and nephroprotective properties. For human nutrition, diet is the major source of tocols (vitamin E) in the body. Thus, there is a need to analyze the different forms of tocols in the diet for the recommendations and to monitor the intake in the body accurately. Several methods have been developed for effective extraction, selective chromatographic separation and sensitive detection of tocols in food. Major advancements also have been made in the field of mass spectrometry for high throughput analysis of primary and secondary metabolites in fruits, vegetables, and grains. This review discusses the theoretical aspects and modern developments in methods of extraction, chromatographic separation, and detection of tocols in plants and their products. Additionally, future research challenges in this perspective are also identified.

http://dx.doi:10.1016/j.foodres.2016.01.025

127. Direct Enantiomer Determination Of Methorphan By HPLC-MS And SFC-MS
2016 - Forensic Chemistry

Keywords: Dextromethorphan, Levomethorphan, High performance liquid chromatograph, Supercritical fluid chromatography, Mass spectrometry, Enantiomer determination

Abstract
The direct and rapid chiral analysis of methorphan was achieved with excellent sensitivity and selectivity using ultra high-performance liquid chromatography-single quadrupole mass spectrometry (HPLC-SQD) and supercritical fluid chromatography-single quadrupole mass spectrometry (SFC-SQD). The optimized separation of dextromethorphan and levomethorphan by HPLC-SQD was accomplished in under 9 min using a Chirobiotic V2 (2.1 × 250 mm, 5 µm) column with a mobile phase consisting of MeOH/NH₄OH/HOAc (100/0.02/0.1, v/v/v). The optimal separation by SFC-SQD was achieved in less than 3 min using a Trefoil AMY1 (2.1 × 150 mm, 2.5 µm) column with a mobile phase comprised of CO₂, 9% MeOH as a co-solvent, and 0.3% cyclohexylamine as a modifier. The limit of detection was 10 ng/mL in the selected ion recording mode for both systems.

http://dx.doi.org/10.1016/j.forc.2016.10.004

2016 - International Journal for Parasitology: Drugs and Drug Resistance

Keywords: Antimalarial, Antiplasmodial, Arylamino alcohol, Plasmepsin II enzyme, Hemozoin inhibition, Mannich reaction, UPC2

Abstract
Synthesis of new 1-aryl-3-substituted propanol derivatives followed by structure-activity relationship, in silico drug-likeness, cytotoxicity, genotoxicity, in silico metabolism, in silico pharmacophore modeling, and in vivo studies led to the identification of compounds 22 and 23 with significant in vitro antiplasmodial activity against drug sensitive (D6 IC$_{50}$ ≤ 0.19 μM) and multidrug resistant (FCR-3 IC$_{50}$ ≤ 0.40 μM and C235 IC$_{50}$ ≤ 0.28 μM) strains of *Plasmodium falciparum*. Adequate selectivity index and absence of genotoxicity was also observed. Notably, compound 22 displays excellent parasitemia reduction (98 ± 1%), and complete cure with all treated mice surviving through the entire period with no signs of toxicity. One important factor is the agreement between in vitro potency and in vivo studies. Target exploration was performed; this chemotype series exhibits an alternative antimalarial mechanism.

http://dx.doi.org/10.1016/j.ijpddr.2016.09.004


2016 - International Journal for Parasitology: Drugs and Drug Resistance

Keywords: Antimalarial, Antiplasmodia, Arylamino alcohol, Plasmepsin II enzyme, Hemozoin inhibition, Mannich reaction, Genotoxicity

Abstract
Synthesis of new 1-aryl-3-substituted propanol derivatives followed by structure-activity relationship, in silico drug-likeness, cytotoxicity, genotoxicity, in silico metabolism, in silico pharmacophore modeling, and in vivo studies led to the identification of compounds 22 and 23 with significant in vitro antiplasmodial activity against drug sensitive (D6 IC$_{50}$ ≤ 0.19 μM) and multidrug resistant (FCR-3 IC$_{50}$ ≤ 0.40 μM and C235 IC$_{50}$ ≤ 0.28 μM) strains of *Plasmodium falciparum*. Adequate selectivity index and absence of genotoxicity was also observed. Notably, compound 22 displays excellent parasitemia reduction (98 ± 1%), and complete cure with all treated mice surviving through the entire period with no signs of toxicity. One important factor is the agreement between in vitro potency and in vivo studies. Target exploration was performed; this chemotype series exhibits an alternative antimalarial mechanism.

http://dx.doi.org/10.1016/j.ijpddr.2016.09.004
130. Screening Of Indian Lingzhi Or Reishi Medicinal Mushroom, *Ganoderma Lucidum* (Agaricomycetes): A UPC²-SQD-MS Approach

**Abstract**

Oriental medicinal mushroom *Ganoderma lucidum* has been widely used for the promotion of health and longevity owing to its various bioactive constituents. Therefore, comprehending metabolomics of different *G. lucidum* parts could be of paramount importance for investigating their pharmacological properties. Ultra-performance convergence chromatography (UPC2) along with mass spectrometry (MS) is an emerging technique that has not yet been applied for metabolite profiling of *G. lucidum*. This study has been undertaken to establish metabolomics of the aqueous extracts of mycelium (GLM), fruiting body (GLF), and their mixture (GLMF) using ultra-performance convergence chromatography single quadrupole mass spectrometry (UPC2-SQD-MS). Aqueous extracts of *G. lucidum* prepared using an accelerated solvent extraction technique have been characterized for their mycochemical activities in terms of total flavonoid content, 1,1-diphenyl-2-picryl-hydrazyl scavenging activity, and ferric ion reducing antioxidant power. The UPC2-SQD-MS technique has been used for the first time for metabolite profiling of *G. lucidum* on a Princeton Diol column (4.6 × 250 mm; 5 µm) using supercritical CO2 (solvent) and 20 mM ammonium acetate in methanol (co-solvent). In the present study, UPC2-SQD-MS was found to be a rapid, efficient, and high-throughput analytical technique, whose coupling to principal component analysis (PCA) and phytochemical evaluation could be used as a powerful tool for elucidating metabolite diversity between mycelium and fruiting body of *G. lucidum*. PCA showed a clear distinction in the metabolite compositions of the samples. Mycochemical studies revealed that overall GLF possessed better antioxidant properties among the aqueous extracts of *G. lucidum*.

http://dx.doi.org/10.1615/IntJMedMushrooms.v18.i2.80

131. Influence Of Uptake Pathways On The Stereoselective Dissipation Of Chiral Neonicotinoid Sulfoxaflor In Greenhouse Vegetables

**Abstract**

Stereoselectivity is of vital importance in our environment and needs to be taken into account for comprehensive risk assessment and regulatory decisions of chiral neonicotinoid sulfoxaflor.
However, little is known about the dissipation of sulfoxaflor stereoisomers with respect to stereoselectivity in plants under greenhouse cultivation. To bridge the knowledge gap, the current study was initiated to investigate the stereoselective degradation of sulfoxaflor in solar greenhouse cucumber and tomato from foliage and root uptake pathways. The stereoselective dissipation of sulfoxaflor was not statistically different between enantiomer pairs from foliage and root pathways of vegetables ($P < 0.05$). The persistence of sulfoxaflor stereoisomers was consistently prolonged under the foliage uptake pathway ($t_{1/2}$, 3.38–14.09 days) compared to the root uptake pathway ($t_{1/2}$, 2.65–5.07 days) in both vegetable fruits. Nevertheless, the concentrations of (+)-sulfoxaflor A and (−)-sulfoxaflor B were both slightly higher than that of their antipode. The tiny difference should be emphasized because it might be magnified to a significant difference by the high-potential bioaccumulation of sulfoxaflor in the food chain.

http://dx.doi.org/10.1021/acs.jafc.5b05940

132. Comparison Of Liquid And Supercritical Fluid Chromatography Mobile Phases For Enantioselective Separations On Polysaccharide Stationary Phases

2016 - Journal of Chromatography A

Keywords: Université d’Orléans, Novartis Institutes for BioMedical Research, active pharmaceutical ingredients, achiral probes, enantiomers, enantiomer separation, racemates pharmaceutical

Abstract

Analysis and production of enantiomerically pure compounds is a major topic of interest when active pharmaceutical ingredients are concerned. Enantioselective chromatography has become a favourite both at the analytical and preparative scales. High-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) are dominating the scene and are often seen as complementary techniques. Nowadays, for economic and ecologic reasons, SFC may be preferred over normal-phase HPLC (NPLC) as it allows significant reductions in solvent consumption. However, the transfer of NPLC methods to SFC is not always straightforward. In this study, we compare the retention of achiral molecules and separation of enantiomers under supercritical fluid (carbon dioxide with ethanol or isopropanol) and liquid normal-phase (heptane with ethanol or isopropanol) elution modes with polysaccharide stationary phases in order to explore the differences between the retention and enantioseparation properties between the two modes. Chemometric methods (namely quantitative structure-retention relationships and discriminant analysis) are employed to compare the results obtained on a large set of analytes (171 achiral probes and 97 racemates) and gain some understanding on the retention and separation mechanisms. The results indicate that, contrary to popular belief, carbon dioxide - solvent SFC mobile phases are often weaker eluents than liquid mobile phases. It appears that SFC and NPLC elution modes provide different retention mechanisms. While some
enantioseparations are unaffected, facilitating the transfer between the two elution modes, other enantioseparations may be drastically different due to different types and strength of interactions contributing to enantioselectivity.

doi:10.1016/j.chroma.2016.06.060

133. Liquid Chromatography And Supercritical Fluid Chromatography As Alternative Techniques To Gas Chromatography For The Rapid Screening Of Anabolic Agents In Urine

Keywords: University of Geneva, Charles University, Universitaire Vaudois and University of Lausanne, anabolic steroids, doping control, health science

Abstract
This work describes the development of two methods involving supported liquid extraction (SLE) sample treatment followed by ultra-high performance liquid chromatography or ultra-high performance supercritical fluid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS and UHPSFC-MS/MS) for the screening of 43 anabolic agents in human urine. After evaluating different stationary phases, a polar-embedded C18 and a diol columns were selected for UHPLC-MS/MS and UHPSFC-MS/MS, respectively. Sample preparation, mobile phases and MS conditions were also finely tuned to achieve highest selectivity, chromatographic resolution and sensitivity. Then, the performance of these two methods was compared to the reference routine procedure for steroid analyses in anti-doping laboratories, which combines liquid-liquid extraction (LLE) followed by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). For this purpose, urine samples spiked with the compounds of interest at five different concentrations were analyzed using the three analytical platforms. The retention and selectivity of the three techniques were very different, ensuring a good complementarity. However, the two new methods displayed numerous advantages. The overall procedure was much faster thanks to high throughput SLE sample treatment using 48-well plates and faster chromatographic analysis. Moreover, the highest sensitivity was attained using UHPLC-MS/MS with 98% of the doping agents detected at the lowest concentration level (0.1ng/mL), against 76% for UHPSFC-MS/MS and only 14% for GC-MS/MS. Finally, the weakest matrix effects were obtained with UHPSFC-MS/MS with 76% of the analytes displaying relative matrix effect between -20 and 20%, while the GC-MS/MS reference method displayed very strong matrix effects (over 100%) for all of the anabolic agents.

doi:10.1016/j.chroma.2016.05.004

134. Ultra-High Performance Supercritical Fluid Chromatography Coupled With Quadrupole-Time-Of-Flight Mass Spectrometry As A Performing Tool For Bioactive Analysis

Keywords: University of Geneva, Nestle Institute of Health Sciences, natural products, plant metabolites, health science

Abstract
Secondary metabolites are an almost unlimited reservoir of potential bioactive compounds. In view of the wide chemical space covered by natural compounds, their comprehensive analysis requires multiple cutting-edge approaches. This study evaluates the applicability of ultra-high performance supercritical fluid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UHPSFC-QqToF-MS) as an analytical strategy for plant metabolites profiling. Versatility of this analytical platform was first assessed using 120 highly diverse natural compounds (according to lipophilicity, hydrogen bond capability, acid-base properties, molecular mass and chemical structure) that were screened on a set of 15 rationally chosen stationary phase chemistries. UHPSFC-QqToF-MS provides a suitable analytical solution for 88% of the tested compounds. Three stationary phases (Diol, not endcapped C18 and 2-EP) were highlighted as particularly polyvalent, since they allow suitable elution of 101 out of 120 natural compounds. The systematic evaluation of retention and selectivity of natural compounds further underlined the suitability of these three columns for the separation of natural compounds. This reduced set of key stationary phases
135. Analysis Of Polar Urinary Metabolites For Metabolic Phenotyping Using Supercritical Fluid Chromatography And Mass Spectrometry

Keywords: King’s College, Imperial College, Waters Corporation, method development, health science

Abstract
Supercritical fluid chromatography (SFC) is frequently used for the analysis and separation of non-polar metabolites, but remains relatively underutilised for the study of polar molecules, even those which pose difficulties with established reversed-phase (RP) or hydrophilic interaction liquid chromatographic (HILIC) methodologies. Here, we present a fast SFC-MS method for the analysis of medium and high-polarity (-7≤cLogP≤2) compounds, designed for implementation in a high-throughput metabonomics setting. Sixty polar analytes were first screened to identify those most suitable for inclusion in chromatographic test mixtures; then, a multi-dimensional method development study was conducted to determine the optimal choice of stationary phase, modifier additive and temperature for the separation of such analytes using SFC. The test mixtures were separated on a total of twelve different column chemistries at three different temperatures, using CO2-methanol-based mobile phases containing a variety of polar additives. Chromatographic performance was evaluated with a particular emphasis on peak capacity, overall resolution, peak distribution and repeatability. The results suggest that a new generation of stationary phases, specifically designed for improved robustness in mixed CO2-methanol mobile phases, can improve peak shape, peak capacity and resolution for all classes of polar analytes. A significant enhancement in chromatographic performance was observed for these urinary metabolites on the majority of the stationary phases when polar additives such as ammonium salts (formate, acetate and hydroxide) were included in the organic modifier, and the use of water or alkylamine additives was found to be beneficial for specific subsets of polar analytes. The utility of these findings was confirmed by the separation of a mixture of polar metabolites in human urine using an optimised 7min gradient SFC method, where the use of the recommended column and co-solvent combination resulted in a significant improvement in chromatographic performance.

doi:10.1016/j.chroma.2016.04.040

136. Selective Enrichment In Bioactive Compound From Kniphofia Uvaria By Super/Subcritical Fluid Extraction And Centrifugal Partition Chromatography

Keywords: Orleans University, LVMH Recherche, anthraquinones, natural products, antioxidants, bioactives, seeds, pharmaceutical

Abstract
Nowadays, a large portion of synthetic products (active cosmetic and therapeutic ingredients) have their origin in natural products. Kniphofia uvaria is a plant from Africa which has proved in the past by in-vivo tests an antioxidant activity due to compounds present in roots. Recently, we have observed anthraquinones in K. uvaria seeds extracts. These derivatives are natural colorants which could have interesting bioactive potential. The aim of this study was to obtain an extract enriched in anthraquinones from K. uvaria seeds which mainly contains glycerides. First, the separation of the seed compounds was studied by using supercritical fluid chromatography (SFC) in the goal to provide a rapid quantification method of these bioactive compounds. A screening of numerous polar stationary phases was achieved for selecting the most suited phase to the separation of the four anthraquinones founded in the seeds. A gradient elution was optimized for improving the separation of the bioactive compounds from the numerous other families of major compounds of the extracts (fatty acids, di- and triglycerides). Besides, a non-selective and green Supercritical Fluid Extraction (SFE) with pure CO2 was applied to seeds followed by a Centrifugal
Partition Chromatography (CPC). The CPC system was optimized by using the Arizona phase system, to enrich the extract in anthraquinones. Two systems were selected to isolate the bioactive compounds from the oily extract with varied purity target. The effect of the injection mode for these very viscous samples was also studied. Finally, in order to directly apply a selective process of extraction to the seeds, the super/subcritical fluid extraction was optimized to increase the anthraquinone yield in the final extract, by studying varied modifier compositions and nature, as well as different temperatures and backpressures. Conditions suited to favour an enrichment factor bases on the ratio of anthraquinone and trilglycerides extracted are described.

doi:10.1016/j.chroma.2016.04.029

137. Evaluation Of Innovative Stationary Phase Ligand Chemistries And Analytical Conditions For The Analysis Of Basic Drugs By Supercritical Fluid Chromatography

Keywords: Geneva, 2-Picolylamine ligand chemistry, 1-Aminoanthracene ligand chemistry, Diethylamine ligand chemistry, pharmaceutical

Abstract

Similar to reversed phase liquid chromatography, basic compounds can be highly challenging to analyze by supercritical fluid chromatography (SFC), as they tend to exhibit poor peak shape, especially those with high pKa values. In this study, three new stationary phase ligand chemistries available in sub -2 μm particle sizes, namely 2-picolylamine (2-PIC), 1-aminoanthracene (1-AA) and diethylamine (DEA), were tested in SFC conditions for the analysis of basic drugs. Due to the basic properties of these ligands, it is expected that the repulsive forces may improve peak shape of basic substances, similarly to the widely used 2-ethylypyridine (2-EP) phase. However, among the 38 tested basic drugs, less of 10% displayed Gaussian peaks (asymmetry between 0.8 and 1.4) using pure CO2/methanol on these phases. The addition of 10mM ammonium formate as mobile phase additive, drastically improved peak shapes and increased this proportion to 67% on 2-PIC. Introducing the additive in the injection solvent rather than in the organic modifier, gave acceptable results for 2-PIC only, with 31% of Gaussian peaks with an average asymmetry of 1.89 for the 38 selected basic drugs. These columns were also compared to hybrid silica (BEH), DIOL and 2-EP stationary phases, commonly employed in SFC. These phases commonly exhibit alternative retention and selectivity. In the end, the two most interesting ligands used as complementary columns were 2-PIC and BEH, as they provided suitable peak shapes for the basic drugs and almost orthogonal selectivities.

doi:10.1016/j.chroma.2016.02.029

138. A Closer Study Of Methanol Adsorption And Its Impact On Solute Retentions In Supercritical Fluid Chromatography

Keywords: Karlstadt University, AstraZeneca, tracer pulse, solute retention factors, separation science

Abstract

Surface excess adsorption isotherms of methanol on a diol silica adsorbent were measured in supercritical fluid chromatography (SFC) using a mixture of methanol and carbon dioxide as mobile phase. The tracer pulse method was used with deuterium labeled methanol as solute and the tracer peaks were detected using APCI-MS over the whole composition range from neat carbon dioxide to neat methanol. The results indicate that a monolayer (4Å) of methanol is formed on the stationary phase. Moreover, the importance of using the set or the actual methanol fractions and volumetric flows in SFC was investigated by measuring the mass flow respective pressure and by calculations of the actual volume fraction of methanol. The result revealed a significant difference between the value set and the actually delivered volumetric methanol flow rate, especially at low modifier fractions. If relying only on the set methanol fraction in the calculations, the methanol layer thickness should in this system be highly overestimated. Finally, retention times for a set of solutes were measured and related to the findings summarized above concerning methanol adsorption. A strongly non-linear relationship between the logarithms of the retention factors and the modifier fraction in the mobile phase was revealed, prior to the established monolayer. At modifier fractions above that required for establishment of the methanol monolayer, this
relationship turns linear which explains why the solute retention factors are less sensitive to changes in modifier content in this region.
doi:10.1016/j.chroma.2016.03.006

139. An Improved Classification Of Stationary Phases For Ultra-High Performance Supercritical Fluid Chromatography

Keywords: University of Orleans, superficially porous particles, ionic interactions, selectivity, solvation parameter model, separation science

Abstract
Supercritical fluid chromatography (SFC) has recently benefited of new instrumentation, together with the availability of many ultra-high performance columns (sub -2μm fully porous particles or sub -3μm superficially porous particles), rendering it more attractive than ever. Most of these columns commonly used in SFC were initially developed for HPLC use, with an increasing number of stationary phases specifically designed for SFC. While the availability of different stationary phase chemistries is an advantage to achieve successful SFC separations, selecting a column for method development remains difficult. For this reason, we have previously developed a classification of stationary phases dedicated to SFC use. It is based on linear solvation energy relationships (LSER) with Abraham descriptors (for neutral species). While current interest in SFC is strong in the pharmaceutical industry, the need to take account of interactions occurring with ionisable species is pressing. We have previously shown how a modified version of the solvation parameter model, adapted to take account of ionic and ionizable species, could be applied to the characterization of SFC systems. In the present paper, based on this modified LSER model, and on the analysis of 109 neutral and ionisable species, we propose an improved classification of 31 ultra-high performance stationary phases to facilitate method development with SFC.
doi:10.1016/j.chroma.2016.02.052

140. Assessment Of Ultra High Performance Supercritical Fluid Chromatography As A Separation Technique For The Analysis Of Seized Drugs: Applicability To Synthetic Cannabinoids

Keywords: George Washington University, Florida International University, positional isomers, health science

Abstract
The recent development of modern methods for ultra high performance supercritical fluid chromatography (UHPSFC) has great potential for impacting the analysis of seized drugs. In the separation of synthetic cannabinoids the technique has the potential to produce superior resolution of positional isomers and diastereomers. To demonstrate this potential we have examined the capability of UHPSFC for the analysis of two different groups of synthetic cannabinoids. The first group was a mixture of 22 controlled synthetic cannabinoids, and the second group included JWH018 and nine of its non-controlled positional isomers The clear superiority of UHPSFC over other separation techniques was demonstrated, in that it was capable of near baseline separation of all 10 positional isomers using a chiral column. In total we examined four achiral columns, including Acquity UPC² Torus 2-PIC, Acquity UPC² Torus Diol, Acquity UPC² Torus DEA and Acquity UPC² Torus 1-AA (1.7μm 3.0×100mm), and three chiral columns, including Acquity UPC² Trefoil AMY1, Acquity UPC² Trefoil CEL1 and Acquity UPC² Trefoil CEL2 (2.5μm 3.0×150mm), using mobile phase compositions that combined carbon dioxide with methanol, acetonitrile, ethanol or isopropanol modifier gradients. Detection was performed using simultaneous PDA UV detection and quadrupole mass spectrometry. The orthogonality of UHPSFC, GC and UHPLC for the analysis of these compounds was demonstrated using principal component analysis. Overall we feel that this new technique should prove useful in the analysis and detection of seized drug samples, and will be a useful addition to the compendium of methods for drug analysis.
doi:10.1016/j.chroma.2016.02.047

141. Separation Of Substrates And Closely Related Glucuronide Metabolites Using Various Chromatographic Modes

Keywords: University of Geneva, University of Lausanne, pharmaceutical
Abstract
A method for the determination of 200 pesticides and pesticide metabolites in honeybee samples has been developed and validated. Almost 98% of compounds included in this method are approved to use within European Union, as active substances of plant protection products or veterinary medicinal products used by beekeepers to control mites Varroa destructor in hives. Many significant metabolites, like metabolites of imidacloprid, thiacloprid, fipronil, methiocarb and amitraz, are also possible to detect. The sample preparation was based on the buffered QuEChERS method. Samples of bees were extracted with acetonitrile containing 1% acetic acid and then subjected to clean-up by dispersive solid phase extraction (dSPE) using a new Z-Sep+ sorbent and PSA. The majority of pesticides, including neonicotinoids and their metabolites, were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) but some of pesticides, especially pyrethroid insecticides, were analyzed by gas chromatography tandem mass spectrometry (GC-MS/MS). The procedure was validated according to the Guidance document SANCO/12571/2013 at four concentration levels: 1, 5, 10 and 100 ng/g bees and verified in the international proficiency test. The analysis of bee samples spiked at the limit of quantification (LOQ) showed about 98% mean recovery value (trueness) and 97% of analytes showed recovery in the required range of 70-120% and RSDr (precision) below 20%. Linearity and matrix effects were also established. The LOQs of pesticides were in the range of 1-100 ng/g. The developed method allows determination of insecticides at concentrations of 10 ng/g or less, except abamectin and tebufenozide. LOQ values are lower than the median lethal doses LD50 for bees. The method was used to investigate more than 70 honeybee poisoning incidents. Data about detected pesticides and their metabolites are included.

doi:10.1016/j.chroma.2016.01.033

142. A Simple, Accurate, Time-Saving And Green Method For The Determination Of 15 Sulfonamides And Metabolites In Serum Samples By Ultra-High Performance Supercritical Fluid Chromatography

Keywords: Chinese Academy of Inspection and Quarantine, Chinese Medical University, pharmaceuticals

Abstract
An analytical method based on ultra-high performance supercritical fluid chromatography (UHPSFC) with photo-diode array detection (PDA) has been developed to quantify 15 sulfonamides and their N4-acetylation metabolites in serum. Under the optimized gradient elution conditions, it took only 7min to separate all 15 sulfonamides and the critical pairs of each parent drug and metabolite were completely separated. Variables affecting the UHPSFC were optimized to get a better separation. The performance of the developed method was evaluated. The UHPSFC method allowed the baseline separation and determination of 15 sulfonamides and metabolites with limit of detection ranging from 0.15 to 0.35μg/mL. Recoveries between 90.1 and 102.2% were obtained with satisfactory precision since relative standard deviations were always below 3%. The proposed method is simple, accurate, time-saving and green, it is applicable to a variety of sulfonamides detection in serum samples.

doi:10.1016/j.chroma.2015.12.075

143. Ultra-Fast High-Efficiency Enantioseparations By Means Of A Teicoplanin-Based Chiral Stationary Phase Made On Sub-2 Totally Porous Silica Particles Of Narrow Size Distribution

Keywords: Sapienza, Ferrara, Sigma Aldrich, chiral carboxylic acids, separation science

Abstract
A new ultra-high performance teicoplanin-based stationary phase was prepared starting from sub-2 μm totally porous silica particles of narrow size distribution. Columns of different lengths were packed at high pressure and a deep and systematic evaluation of kinetic performance, in terms of van Deemter analysis, was performed under different elution conditions (HILIC, POM, RP and NP) by using both achiral and chiral probes. For the achiral probes, the efficiency of the columns at the minimum of the van Deemter curves were very high leading to some 278,000, 270,000, 262,000 and 232,000 plates/m in hydrophilic interaction liquid chromatography (HILIC), polar organic mode (POM), normal phase (NP) and reversed phase (RP) respectively. The lowest plate height,
H_{min}=3.59 \, \mu \text{m (h(/)=1.89)}, was obtained under HILIC conditions at a flow rate of 1.4 mL/min. Efficiency as high as 200,000-250,000 plates/m (at the optimum flow rate) was obtained in the separation of the enantiomers of chiral probes under HILIC/POM conditions. N-protected amino acids, \( \alpha \)-aryloxy acids, herbicides, anti-inflammatory agents were baseline separated on short (2-cm) and ultra-short (1-cm) columns, with analysis time in the order of 1 min. The enantiomers of N-BOC-d,l-methionine were successfully baseline separated in only 11s in HILIC mode. Several examples of fast and efficient resolutions in sub/supercritical fluid chromatography were also obtained for a range of chiral carboxylic acids.

doi:10.1016/j.chroma.2015.11.071

144. Supercritical Fluid Chromatography Coupled With In-Source Atmospheric Pressure Ionization Hydrogen/Deuterium Exchange Mass Spectrometry For Compound Speciation

Keywords: Kyungpook National University, hydrogen/deuterium exchange, hdx, separation science

Abstract
An experimental setup for the speciation of compounds by hydrogen/deuterium exchange (HDX) with atmospheric pressure ionization while performing chromatographic separation is presented. The proposed experimental setup combines the high performance supercritical fluid chromatography (SFC) system that can be readily used as an inlet for mass spectrometry (MS) and atmospheric pressure photo ionization (APPI) or atmospheric pressure chemical ionization (APCI) HDX. This combination overcomes the limitation of an approach using conventional liquid chromatography (LC) by minimizing the amount of deuterium solvents used for separation. In the SFC separation, supercritical CO2 was used as a major component of the mobile phase, and methanol was used as a minor co-solvent. By using deuterated methanol (CH3OD), AP HDX was achieved during SFC separation. To prove the concept, thirty one nitrogen- and/or oxygen-containing standard compounds were analyzed by SFC-AP HDX MS. The compounds were successfully speciated from the obtained SFC-MS spectra. The exchange ions were observed with as low as 1% of CH3OD in the mobile phase, and separation could be performed within approximately 20min using approximately 0.24mL of CH3OD. The results showed that SFC separation and APPI/APCI HDX could be successfully performed using the suggested method.

doi:10.1016/j.chroma.2016.03.011

145. Advances In High-Throughput And High-Efficiency Chiral Liquid Chromatographic Separations

Keywords: The University of Texas at Arlington, High-efficiency chiral separations, High-throughput chromatography, Superficially porous particles, HPLC and SFC enantiomeric separations, Ultrafast separations, Instrument optimizations

Abstract:
The need for improved liquid chromatographic chiral separations has led to the advancement of chiral screening techniques as well as the development of new, high efficiency chiral separation methods and stationary phases. This review covers these advancements, which primarily occurred over the last 15 years. High throughput techniques include multi-column screening units, multiple injection sequences, and fast gradient SFC screening. New separation methods and column technologies that aim at high efficiency chiral separations include the use of achiral UHPLC (i.e. sub-2 \, \mu \text{m}) columns for separating derivatized chiral analytes or using chiral additives in the run buffer, UHPLC chiral stationary phases, and superficially porous particle based chiral stationary phases. Finally, the enhancement of chiral separations through these new technologies requires that certain instrumental considerations be made. Future directions in continuing to improve chiral separations are also discussed.

http://dx.doi.org/10.1016/j.chroma.2016.07.040
146. Rapid Characterization Of Commercial Polysorbate 80 By Ultra-High Performance Supercritical Fluid Chromatography Combined With Quadrupole Time-Of-Flight Mass Spectrometry

Keywords: UHPSFC-QTOF-MS, Polysorbate 80, Retention model

Abstract:
Polysorbate 80, as a nonionic surfactant, is widely used in the food, personal care, and pharmaceutical industries due to the advantages of high surface activity, low toxicity, etc. In fact, the products of polysorbate 80 are complex mixtures of oligomers. In this work, a novel and fast method was developed to characterize the commercial polysorbate 80 by ultra-high performance supercritical fluid chromatography (UHPSFC) combined with quadrupole time-of-flight mass spectrometry (QTOF-MS). Some crucial parameters, such as temperature, back pressure and flow rate were optimized. UHPSFC could distinguish n-mer from (n − 1)-mer and (n + 1)-mer in the same series, which provided the high separation resolution needed for quantitative determination of each oligomer in same series. It was not achieved in previous studies. Furthermore, the characteristic ion fragments were found in MS/MS experiment and used to identify different series. The results revealed that main components of this nonionic surfactant comprise polyethylene oxide (PEO), PEO-monooleate, PEO-isosorbide, PEO-isosorbide monooleate, PEO-isosorbide dioleate, PEO-sorbitan, PEO-sorbitan monooleate, PEO-sorbitan dioleate and PEO-sorbitan trioleate, etc. The separation was performed using BEH stationary phase, so the relationship between molecular structure of these oligomers and chromatographic retention behavior in supercritical fluid chromatography were also investigated for first time. The whole analytical process only takes 8 min for one sample. Therefore, UHPSFC-QTOF-MS is a simple, novel and efficient tool to analyze polysorbate 80.

http://dx.doi.org/10.1016/j.chroma.2016.08.051

147. Maximizing Performance In Supercritical Fluid Chromatography Using Low-Density Mobile Phases

Keywords: Supercritical fluid chromatography, Low-density carbon dioxide, Column efficiency, Vacuum technology, Decoupling between inlet eluent temperature and oven temperature, Insulating material

Abstract
The performance of a 3.0 mm × 150 mm column packed with 1.8 μm fully porous HSS-SB-C18 particles was investigated in supercritical fluid chromatography (SFC) with low-density, highly expandible carbon dioxide. These conditions are selected for the analysis of semi-volatile compounds. Elevated temperatures (>100 °C) were then combined with low column back pressures (<100 bar). In this work, the inlet temperature of pure carbon dioxide was set at 107 °C, the active back pressure regulator (ABPR) pressure was fixed at 100 bar, and the flow rate was set at 2.1 mL/min at 12 °C (liquefied carbon dioxide) and at an inlet column pressure close to 300 bar. Nine n-alkylbenzenes (from benzene to octadecylbenzene) were injected under linear (no sample overload) conditions. The severe steepness of the temperature gradients across the column diameter were predicted from a simplified heat transfer model. Such conditions dramatically lower the column performance by affecting the symmetry of the peak shape. In order to cope with this problem, three different approaches were experimentally tested. They include (1) the decoupling and the proper selection of the inlet eluent temperature with respect to the oven temperature, (2) the partial thermal insulation of the column using polyethylene aerogel, and (3) the application of a high vacuum (10−5 Torr provided by a turbo-molecular pump) in a housing chamber surrounding the whole column body.

The results reveal that (1) the column efficiency can be maximized by properly selecting the difference between the eluent and the oven temperatures, (2) the mere wrapping of the column with an excellent insulating material is insufficient to fully eliminate heat exchanges by conduction and the undesirable radial density gradients across the column i.d., and (3) the complete thermal insulation of the SFC column under high vacuum allows to maximize the column efficiency by maintaining the integrity of the peak shape.

http://dx.doi.org/10.1016/j.chroma.2016.09.024
148. Unexpected Retention And Efficiency Behaviors In Supercritical Fluid Chromatography: A Thermodynamic Interpretation

Keywords: Supercritical fluid chromatography, Sample solvent effects, Retention shift, Band compression, Band enlargement

Abstract

Experimental conditions leading to unexpected shift in retention, band compression, and to band enlargement of small molecules in supercritical fluid chromatography are reported. The stationary phase is a 3.0 mm x 150 mm column packed with 1.8 μm fully porous high strength silica (HSS) StableBond (SB) C18 particles. The mobile phase is pure carbon dioxide preheated at 107 °C and the column back pressure is set at 100 bar. The column was thermally insulated in a vacuum chamber at a pressure of 10^{-3} Torr in order to maintain the integrity of the peak symmetry. The sample solution was prepared by dissolving seven n-alkylbenzenes (from benzene to dodecylbenzene) in pure acetonitrile. The injected sample volume (1 μL) was three orders of magnitude smaller than the column volume. Remarkably, the retention time of octylbenzene is found 15% smaller than that expected for this series of homologous compounds. Most strikingly, the plate counts change from about 20 000 for the three least retained analytes (benzene, ethylbenzene, and butylbenzene) to 60 000 for hexylbenzene and to only 5000 for the three most retained compounds (octylbenzene, decylbenzene, and dodecylbenzene). These unexpectedly high (reduced plate height of 1.3) and low (reduced plate height of 15) column efficiencies observed for closely related compounds are consistent with the overlap between the spatial concentration zone of the sample solvent (acetonitrile, Langmuir isotherm, \( k \approx 2 \)) and those of the analytes (competitive linear isotherms, \( 0 < k < 10 \)). The present observations are fully supported by chromatogram simulations which assume that the Henry’s constants of the infinitely diluted analytes are strongly dependent on the concentration of the sample solvent in the mobile phase.

http://dx.doi.org/10.1016/j.chroma.2016.09.020

149. Peak Deformations In Preparative Supercritical Fluid Chromatography Due To Co-Solvent Adsorption

Keywords: Supercritical fluid chromatography, SFC, Solvent adsorption, Adsorption strength, Langmuir band shape, Anti-Langmuir band shape

Abstract:

In supercritical fluid chromatography (SFC) the mobile phase comprises of carbon dioxide (CO2) as main solvent and smaller amounts of an organic polar solvent (often an alcohol) as co-solvent. The co-solvent is considered to function by changing the overall polarity of the eluent, i.e. by acting as a “modifier”. However, recent studies indicate that the co-solvent methanol can also adsorb to some common SFC stationary phases. Hence, the co-solvent should also be able to function as an “adsorbing additive”, i.e. an eluent component that competes with the injected solutes about the stationary phase surface. In this study it was found by fitting different mechanistic models to systematic experimental data, that the co-solvent methanol can have both functions: at low co-solvent fractions, methanol acts as an additive whereas at larger fractions it acts as a modifier. Moreover, it was found that when the co-solvent adsorbs more strongly to the stationary phase than the solute, “bizarre” deformations of the preparative band shapes can occur. This is illustrated by a solute that converts from a normal “Langmuirian” band shape to an “anti-Langmuirian” shape when changing from neat carbon dioxide (CO2) to an eluent containing co-solvent. This peak shape transition is dependent on both (i) the relative retention of the solute and co-solvent to the stationary phase in eluent containing neat CO2 and on (ii) the relative retention of the additive perturbation peak and the solute peak in eluent containing also co-solvent.

http://dx.doi.org/10.1016/j.chroma.2016.09.019

150. Comparison Of Ultra-High Performance Methods In Liquid And Supercritical Fluid Chromatography Coupled To Electrospray Ionization – Mass Spectrometry For Impurity Profiling Of Drug Candidates

Keywords: High-resolution separations, Impurity profiling, Orthogonal methods, Performance comparison, Pharmaceutical ingredients

Abstract:
Impurity profiling of organic products synthesized as possible drug candidates represents a major analytical challenge. Complementary analytical methods are required to ensure that all impurities are detected. Both high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) can be used for this purpose.

In this study, we compared ultra-high performance HPLC (UHPLC) and ultra-high performance SFC (UHPSFC) using a large dataset of 140 pharmaceutical compounds. Four previously optimized methods (two on each technique) were selected to ensure fast high-resolution separations. The four methods were evaluated based on response rate, peak capacity, peak shape and capability to detect impurities (UV). The orthogonality between all methods was also assessed. The best UHPLC method and UHPSFC methods provided comparable quality for the 140 compounds included in this study. Moreover, they were found to be highly orthogonal. At last, the potential of the combined use of UHPLC and UHPSFC for impurity profiling is illustrated with practical examples.

http://dx.doi.org/10.1016/j.chroma.2016.10.045

151. Bridging The Gap Between Gas And Liquid Chromatography
2016 - Journal of Chromatography A

Keywords: Low-density fluid chromatography, Supercritical fluid chromatography, Vacuum technology, Carbon dioxide, Volatile and non-volatile compounds, Fast and high-resolution separation

Abstract:
The rapid and complete baseline separation of both volatile (C5 to C16 alkanes in gasoline or terpenes in plant extracts) and non-volatile (>C20 alkanes) organic compounds was achieved by combining (1) low-density fluid chromatography (LDFC) using carbon dioxide at elevated temperature (>90 °C) and low pressure (1500 psi) designed to increase the retention of the most volatile compounds and (2) high-vacuum technology (<10^-4 Torr) in order to preserve the maximum efficiency of short analytical columns (3.0 mm × 150 mm packed with 1.8 μm fully porous HSS-SB-C18 particles) when used in LDFC. The volatile compounds are eluted first under isobaric conditions (1500 psi) in less than a minute followed by a linear gradient of the column back pressure (from 1500 to 3500 psi in 5 min) for the elution of the non-volatile compounds up to C40. The experimental results demonstrate that LDFC performed with short 3.0 mm i.d. columns packed with sub-2 μm particles and placed under adiabatic conditions enables the analysts to deliver a single, fast, and high-resolution separation of both volatile and non-volatile compounds.

http://dx.doi.org/10.1016/j.chroma.2016.10.038

152. Development Of Separation Methods For The Chiral Resolution Of Hexahelicenes
2016 - Journal of Chromatography A

Keywords: Helicene, P/M enantiomer, Chirality, High-performance liquid chromatography, Supercritical fluid chromatography, Capillary electrophoresis

Abstract:
In this short communication we report optimized procedures for the chiral separation of non-charged [6]helicene (1) and cationic derivative 1-butyl-3-(2-methyl[6]helicenyl)-imidazolium bromide (2) using high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) methods. The possibility of using capillary electrophoresis (CE) was also tested. The satisfactory results were obtained with SFC, where the highly selective resolution of four enantiopure 1 and 2 helicenes was achieved in a single run within 5 min. The semi-preparative procedure for the isolation of P and M enantiomers of compound 2, including circular dichroism data, is reported here for the first time. The results could be used in further separations and analytical applications targeting carbohelicenes vs. positively charged helicene derivatives.

http://dx.doi.org/10.1016/j.chroma.2016.10.083

153. A Case Of Z/E-Isomers Elution Order Inversion Caused By Cosolvent Percentage Change In Supercritical Fluid Chromatography
2016 - Journal of Chromatography A

Keywords: Supercritical fluid chromatography, Retention mechanisms, Z/E-isomers, Elution order inversion, Modifier adsorption

Abstract:
A case of elution order inversion caused by cosolvent percentage change in supercritical fluid chromatography was observed and investigated in some detail. Z- and E-isomers of phenylisobutyrlketone oxime experience an elution order reversal on most columns if the mobile phase consists of CO2 and alcohol. At lower percentages of alcohol Z-oxime is retained less, somewhere at 2–5% coelution occurs and at larger cosolvent volume elution order reverses – Z-oxime is eluted later than E-oxime. We suppose inversion with CO2-ROH phases happens due to a shift in balance between two main interactions governing retention. At low ROH percentages stationary phase surface is only slightly covered by ROH molecules so oximes primarily interact with adsorption sites via hydrogen bond formation. Due to intramolecular sterical hindrance Z-oxime is less able to form hydrogen bonds and consequently is eluted first. At higher percentages alcohols occupy most of strong hydrogen bonding sites on silica surface thus leaving non-specific electrostatic interactions predominantly responsible for Z/E selectivity. Z-oxime has a much larger dipole moment than E-oxime and at these conditions it is eluted later. Additional experimental data with CO2-CH3CN, hexane-iPrOH and CHF3-ROH mobile phases supporting this explanation are presented.

http://dx.doi.org/10.1016/j.chroma.2016.11.037

154. Enantioseparation And Determination Of Isofenphos-Methyl Enantiomers In Wheat, Corn, Peanut And Soil With Supercritical Fluid Chromatography/Tandem Mass Spectrometric Method

Keywords: State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, QuEChERs, food

Abstract
Supercritical fluid chromatography/tandem mass spectrometry (SFC-MS/MS) is an effective tool in separation science which uses the nontoxic CO2 fluid for better control of analyte retention. Also the technology of a postcolumn additive to complement MS/MS ensure the high-selectivity determination. In this paper, a green and sensitive analytical method was developed for the enantioselective separation and determination of isofenphos-methyl enantiomers in foodstuff and soil by SFC-MS/MS. The enantioseparation was performed within 3.50 min using Chiralpak IA-3 column with CO2/isopropanol (90:10, v/v) as the mobile phase at 2.2 mL/min flow rate. The postcolumn compensation technology provided with 0.1% formic acid/methanol greatly improved the ionization efficiency of mass spectrometry. Column temperature, auto back pressure regulator pressure, and flow rate of compensation solvent were optimized to 30 °C, 2200 psi, and 0.1 mL/min, respectively. The QuEChERs method was adopted in this study, which mean recoveries of isofenphos-methyl enantiomers ranged from 75.7% to 111.4%, with relative standard deviations less than 11.3% at three concentration levels in all matrices. The limits of detection for both enantiomers varied from 0.02 μg/kg to 0.15 μg/kg, while the limit of quantification did not exceed 0.50 μg/kg. The proposed method was then successfully applied to analyze authentic samples, confirming that it was a versatile strategy for the analysis of isofenphos-methyl enantiomers in food and environmental matrices.

doi:10.1016/j.jchromb.2016.02.003

155. Development And Validation Of An Enantioselective SFC-MS/MS Method For Simultaneous Separation And Quantification Of Oxcarbazepine And Its Chiral Metabolites In Beagle Dog Plasma

Keywords: Jilin University, University of Otago, Oxcarbazepine, S-Licarbazepine; R-Licarbazepine, chiral analysis, health science

Abstract
A rapid and sensitive assay based on supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) has been developed and validated for the determination of oxcarbazepine (OXC) and its chiral metabolite licarbazine (Lic) in beagle dog plasma using carbamazepine as internal standard. Chiral analysis in a run time of only 3min was performed on an ACQUITY UPC(2) ™ Trefoill™ CEL2 column (3.0×150mm, 2.5μm) at 50°C by isocratic elution with a mobile phase of supercritical carbon dioxide (purity≥99.99%) and methanol (60:40, v/v) at a flow rate of 2.3mL/min. The assay was linear over the concentration ranges 5-1000ng/mL for OXC and 0.5-100ng/mL for the enantiomers of Lic with corresponding lower limits of quantitation.
of 5ng/mL and 0.5ng/mL. Intra- and inter-day precisions were in the range 0.78-14.14% with accuracies in the range -10.80% to 0.42%. The method was successfully applied to a pharmacokinetic study involving a single oral administration of 16mg/kg OXC as Trileptal® tablets to beagle dogs.

doi:10.1016/j.jchromb.2016.03.013

156. Overcoming Bioanalytical Challenges Associated With The Separation And Quantitation Of GSK1278863, A HIF-Prolyl Hydroxylase Inhibitor And Its 14 Stereoisomeric Metabolites

Keywords: GSK, GlaxoSmithKline, anemia, kidney disease, health science

Abstract
GSK1278863 is an investigative drug under investigation for treatment of anemia associated with chronic kidney disease. Its metabolism is primarily metabolized by P450 enzymes where 19 unique metabolic species have been identified. These include multiple products of mono-, di-, and tri-oxygenation. Initially, two separate and complex ultra high performance liquid chromatography (UHPLC) reverse phase methodologies were developed, validated and applied to measure parent and various predominant and circulating metabolites in numerous clinical studies. However, 5 of the 6 oxidative metabolites may exist in different stereoisomeric forms, resulting in 14 separate species; therefore a chiral methodology was required to determine which stereoisomeric forms circulated in human. A variety of conventional approaches were explored, where in the end a supercritical fluid chromatography (SFC) method was required to separate this complex mixture of 14 stereoisomeric metabolites; data from these experiments provided important information on which species circulate in human. The details of these methodologies will be discussed herein.

http://dx.doi.org/10.1016/j.jchromb.2015.11.057

157. An Improvement Of Separation And Response Applying Post-Column Compensation And One-Step Acetone Protein Precipitation For The Determination Of Coenzyme Q10 In Rat Plasma By SFC-MS/MS

Keywords: Shenyang Pharmaceutical University, Coenzyme Q10, Post-column compensation, SFC-ESI–MS/MS, Pharmacokinetic study, Rat plasma

Abstract
Coenzyme Q10 (CoQ10) solid dispersion was prepared to improve its oral bioavailability due to the poor solubility of CoQ10. To evaluate the pharmacokinetic behaviors of CoQ10 solid dispersion, a simple, rapid, sensitive and environment friendly method for the determination of CoQ10 in rat plasma was developed. In this study, samples were prepared by one-step protein precipitation with acetone and then the supercritical fluid chromatography-electrospray ionization tandem mass spectrometry (SFC-ESI–MS/MS) method was used. The separation was achieved by an ACQUITY UPC²™ BEH 2-EP column (100 mm × 3 mm, 1.7 μm) maintained at 35 °C, using carbon dioxide (≥99.99%) and methanol (85:15, v/v) as the mobile phase at a flow rate of 1.0 ml/min. To improve the response and sensitivity, the compensation solvent of methanol with 2 mM ammonium acetate at a flow rate of 0.2 ml/min was used and the total analysis time was only 1.5 min for each sample. The detection was carried out on a tandem mass spectrometer with electrospray ionization (ESI) and the mass transition ion pair was m/z881.0 → 197.0 and 285.1 → 193.0 for CoQ10 and diazepam, internal standard (IS), respectively. Calibration curve was linear over the concentration range of 2.00–500.00 ng/ml (r² ≥ 0.998) with a lower limit of quantification of 2.00 ng/ml. The intra- and inter-day accuracy and precision were below 15% for all quality control samples. The proposed method was rapid, accurate and reproducible, which was suitable to compare the pharmacokinetic behaviors in rats after a single oral dose of 100 mg/kg CoQ10 solid dispersion or tablets.

http://dx.doi.org/10.1016/j.jchromb.2016.07.050

158. High-Throughput Analysis Of 19 Endogenous Androgenic Steroids By Ultra-Performance Convergence Chromatography Tandem Mass Spectrometry

2016 - Journal of Chromatography B
Keywords: Stellenbosch University, University of Birmingham, Adrenal androgens, Gas chromatography, Steroids, Supercritical fluid chromatography, Ultra performance convergence chromatography, Ultra performance liquid chromatography

Abstract:
11-Oxygenated steroids such as 11-ketotestosterone and 11-ketodihydrotestosterone have recently been shown to play a putative role in the development and progression of castration resistant prostate cancer. In this study we report on the development of a high throughput ultra-performance convergence chromatography tandem mass spectrometry (UPC\textsuperscript{2}-MS/MS) method for the analysis of thirteen 11-oxygenated and six canonical C19 steroids isolated from a cell culture matrix. Using an Acquity UPC\textsuperscript{2} BEH 2-EP column we found that UPC\textsuperscript{2} resulted in superior selectivity, increased chromatographic efficiency and a scattered elution order when compared to conventional reverse phase ultra-performance liquid chromatography (UPLC). Furthermore, there was a significant improvement in sensitivity (5–50 times). The lower limits of quantification ranged between 0.01–10 ng mL\textsuperscript{−1}, while the upper limit of quantification was 100 ng mL\textsuperscript{−1} for all steroids. Accuracy, precision, intra-day variation, recovery, matrix effects and process efficiency were all evaluated and found to be within acceptable limits. Taken together we show that the increased power of UPC\textsuperscript{2}-MS/MS allows the analyst to complete \textit{in vitro} assays at biologically relevant concentrations for the first time and in so doing determine the routes of steroid metabolism which is vital for studies of androgen responsive cancers, such as prostate cancer, and could highlight new mechanisms of disease progression and new targets for cancer therapy.

http://dx.doi.org/10.1016/j.jchromb.2016.07.024

159. Hydroxypyridyl Imines: Enhancing Chromatographic Separation And Stereochemical Analysis Of Chiral Amines Via Circular Dichroism

Keywords: Chiral Amines, imine-bond formation, amino alcohols, stereocenters

Abstract
Imine-bond formation between chiral amines and commercially available 3-hydroxypyridine-2-carboxaldehyde (HCA) was exploited for rapid determination of stereochemical composition. Chiral supercritical fluid chromatography (SFC) screening of the derivatized imine compounds led to the elucidation of multiple combinations of mobile and stationary phases that gave resolution of all members of a series of chiral amines. The first eluting enantiomer was generally the derivative of the (\textit{R})-amine enantiomer across the series that was studied, indicating that the imine formed from the (\textit{S})-amine has more favorable interaction with the chiral stationary phase of the column. These conditions were then applied to more challenging compounds, namely amino alcohols and diastereomers possessing more than one stereocenter. The approach was utilized to monitor stereoselective biocatalytic transamination and assign the absolute configuration of the enantiomeric products. Finally, hydrolysis of the imine bond of the derivative was shown to generate enantiopure amine starting materials without racemization. This further highlights the value of this approach for creating readily reversed derivatives that enhance chromatographic separation and aid in the determination of absolute configuration.

http://dx.doi.org/10.1021/acs.joc.6b01162
Development, Validation And Comparison Of UHPSFC And UHPLC Methods For The Determination Of Agomelatine And Its Impurities

2016 - Journal of Pharmaceutical and Biomedical Analysis

Keywords: Charles University, Zentiva, k.s. Praha (a Sanofi company), antidepressants, pharmaceuticals

Abstract
Agomelatine is one of the newest antidepressants. Due to a different mechanism of action it offers a completely new approach in the treatment of depressive disorders. Two chromatographic methods for determination of agomelatine and its impurities were developed. The separations on UHPSFC system were accomplished using stationary phase based on BEH 2-EP and gradient elution with CO2 and methanol containing 20mM ammonium formate and 5% of water. The UHPLC separations were performed on stationary phase BEH Shield RP18. The mixture of acetonitrile and methanol in ratio 1:1 and ammonium acetate buffer pH 9.5 were used as mobile phase. Both developed methods were properly validated in terms of linearity, sensitivity (LOD, LOQ), accuracy and precision according to ICH guidelines. The UHPSFC method was linear in the range 0.25-70μg/ml for all analytes with method accuracy ≥97.4% and ≥100.2% and method intra-day precision RSD ≤2.4 and ≤0.8 for impurities and API (active pharmaceutical ingredient), respectively. The UHPLC method was linear in the range 0.1-10μg/ml for all analytes except three impurities for which the linear range was larger 0.1-25μg/ml. Method accuracy ≥95.7% and ≥95.2% and method intra-day precision RSD ≤2.6 and ≤1.5 were found for impurities and API, respectively. The measurement of tablet samples was performed and the selected parameters of the methods were compared. In conclusion, both methods were appropriate for the determination of agomelatine and its impurities in pharmaceutical quality control (QC), although the UHPSFC method was found as more convenient especially in the method development phase. The advantages of newly developed UHPSFC-PDA (photo diode array detector) method were its environmental friendliness due to the mobile phase used, a very good resolution, selectivity and high speed of analysis (total time of separation was 4.1min).

Screening Study Of SFC Critical Method Parameters For The Determination Of Pharmaceutical Compounds

161. 2016 - Journal of Pharmaceutical and Biomedical Analysis

Keywords: University of Liege, Arlenda s.a., antimalarial molecules, pharmaceuticals, separation science, method development

Abstract

Nowadays, supercritical fluid chromatography is commonly presented as a promising alternative technique in the field of separation sciences. Nevertheless, the selection of chromatographic conditions and sample preparation of pharmaceutical compounds remain a challenge and peak distortion was previously highlighted. The main objective of the present work was to evaluate the impact of different critical method parameters (CMPs), i.e. stationary phase, mobile phase composition and injection solvent nature. The experiments were performed considering two groups of antimalarial molecules: one group with neutral/apolar compounds and the other one with salt form of polar compounds. In this context, another objective was to propose a suitable sample solvent for quantitative analysis. The interest of new generation stationary phase to obtain good peak shape and the interest to tune the mobile phase composition were demonstrated. During this study, design of experiments and desirability function approach enabled to highlight optimal chromatographic conditions in order to maximise peak capacity and to get acceptable value of symmetry factor. Regarding sample injection solvent composition, some counterintuitive results were observed: solvents closer to the mobile phase polarity (i.e. heptane or 2-propanol/heptane mixture) did not provide best results in terms of peak symmetry. In addition, acetonitrile and short aliphatic alcohols offered an interesting alternative as injection solvent: toxicity of solvents used is clearly reduced and better quantitative performances could be expected while keeping high peak capacity and symmetric sharp peaks. Finally, the quantitative performances were evaluated by the method validation for the quantitative determination of quinine sulfate in a pharmaceutical formulation. These better understandings on critical method parameters led SFC to be an even more promising technique in the field of the analysis of pharmaceutical compounds.

doi:10.1016/j.jpba.2016.04.005
162. Chiral Separations Of Cathionone And Amphetamine-Derivatives: Comparative Study Between Capillary Electrochromatography, Supercritical Fluid Chromatography And Three Liquid Chromatographic Modes

2016 - Journal of Pharmaceutical and Biomedical Analysis

Keywords: Vrije Universiteit Brussel-VUB, Karl-Franzens-Universitat Graz, Tbilisi State University, pharmaceutical

Abstract
The screening part of an earlier defined chiral separation strategy in capillary electrochromatography (CEC) was used for the separation of ten cathinone- and amphetamine derivatives. They were analyzed using 4 polysaccharide-based chiral stationary phases (CSPs), containing cellulose tris(3,5-dimethylphenylcarbamate) (ODRH), amylose tris(3,5-dimethylphenylcarbamate) (ADH), amylose tris(5-chloro-2-methylphenylcarbamate) (LA2), and cellulose tris(4-chloro-3-methylphenylcarbamate) (LC4) as chiral selectors. After applying the screening to each compound, ADH and LC4 showed the highest success rate. In a second part of the study, a comparison between CEC and other analytical techniques used for chiral separations i.e., supercritical fluid chromatography (SFC), polar organic solvent chromatography (POSC), reversed-phase (RPLC) and normal-phase liquid chromatography (NPLC), was made. For this purpose, earlier defined screening approaches for each technique were applied to separate the 10 test substances. This allowed an overall comparison of the success rates of the screening steps of the 5 techniques for these compounds. The results showed that CEC had a similar enantioselectivity rate as NPLC and RPLC, producing the highest number of separations (9 out of 10 racemates). SFC resolved 7 compounds, while POSC gave only 2 separations. On the other hand, the baseline separation success rates for NPLC and RPLC was better than for CEC. For a second comparison, the same chiral stationary phases as in the CEC screening were also tested with all techniques at their specific screening conditions, which allowed a direct comparison of the performance of CEC versus the same CSPs in the other techniques. This comparison revealed that RPLC was able to separate all tested compounds, and also produced the highest number of baseline separations on the CSP that were used in the CEC screening step. CEC and NPLC showed the same success rate: nine out of ten substances were separated. When CEC and NPLC are combined, separation of the ten compounds can be achieved. SFC and POSC resolved eight and three compounds, respectively. POSC was the least attractive option as it expressed only limited enantioselectivity toward these compounds.

doi:10.1016/j.jpba.2015.12.007
163.Comparison Of Ultra-High-Performance Supercritical Fluid Chromatography And Ultra-High-Performance Liquid Chromatography For The Separation Of Spirostanol Saponins

2016 - Journal of Pharmaceutical and Biomedical Analysis

Keywords: Beijing Institute of Radiation Medicine, Waters, Ovation Health Science and Technology Co. Ltd., herbal medicines, isolation, purification, tcm, natural products, health science

Abstract
Spirostanol saponins are important active components of some herb medicines, and their isolation and purification are crucial for the research and development of traditional Chinese medicines. We aimed to compare the separation of spirostanol saponins by ultra-high performance supercritical fluid chromatography (UHPSFC) and ultra-high performance liquid chromatography (UHPLC). Four groups of spirostanol saponins were separated respectively by UHPSFC and UHPLC. After optimization, UHPSFC was performed with a HSS C18 SB column or a Diol column and with methanol as the co-solvent. A BEH C18 column and mobile phase containing water (with 0.1% formic acid) and acetonitrile were used in UHPLC. We found that UHPSFC could be performed automatically and quickly. It is effective in separating the spirostanol saponins which share the same aglycone and vary in sugar chains, and is very sensitive to the number and the position of hydroxyl groups in aglycones. However, the resolution of spirostanol saponins with different aglycones and the same sugar moiety by UHPSFC was not ideal and could be resolved by UHPLC instead. UHPLC is good at differentiating the variation in aglycones, and is influenced by double bonds in aglycones. Therefore, UHPLC and UHPSFC are complementary in separating spirostanol saponins. Considering the naturally produced spirostanol saponins in herb medicines are different both in aglycones and in sugar chains, a better separation can be achieved by combination of UHPLC and UHPSFC. UHPSFC is a powerful technique for improving the resolution when UHPLC cannot resolve a mixture of spirostanol saponins and vice versa.

164. Supercritical Fluid Chromatography For GMP Analysis In Support Of Pharmaceutical Development And Manufacturing Activities

Keywords: Merck, enantiomers, gmp, impurities, chiral stationary phases, method validation, pharmaceutical

Abstract

Supercritical fluid chromatography (SFC) has long been a preferred method for enantiopurity analysis in support of pharmaceutical discovery and development, but implementation of the technique in regulated GMP laboratories has been somewhat slow, owing to limitations in instrument sensitivity, reproducibility, accuracy and robustness. In recent years, commercialization of next generation analytical SFC instrumentation has addressed previous shortcomings, making the technique better suited for GMP analysis. In this study we investigate the use of modern SFC for enantiopurity analysis of several pharmaceutical intermediates and compare the results with the conventional HPLC approaches historically used for analysis in a GMP setting. The findings clearly illustrate that modern SFC now exhibits improved precision, reproducibility, accuracy and robustness; also providing superior resolution and peak capacity compared to HPLC. Based on these findings, the use of modern chiral SFC is recommended for GMP studies of stereochemistry in pharmaceutical development and manufacturing.

doi:10.1016/j.jpba.2015.09.014

165. Supercritical Fluid Chromatography For Separation And Preparation Of Tautomeric 7-Epimeric Spiro Oxindole Alkaloids From Uncaria Macrophylla

Keywords: Spiro oxindole alkaloid, Uncaria macrophylla, Isomerization, Ultra-performance convergence chromatography, Preparative scale supercritical fluid chromatography

Abstract:

Increasing challenge arising from configurational interconversion in aqueous solvent renders it rather difficult to isolate high-purity tautomeric reference standards and thus largely hinders the holistic quality control of traditional Chinese medicine (TCM). Spiro oxindole alkaloids (SOAs), as the markers for the medicinal Uncaria herbs, can easily isomerize in polar or aqueous solvent via a retro-Mannich reaction. In the present study, supercritical fluid chromatography (SFC) is utilized to separate and isolate two pairs of 7-epimeric SOAs, including rhynchophylline (R) and isorhynchophylline (IR), corynoxine (C) and corynoxine B (CB), from Uncaria macrophylla. Initially, the solvent that can stabilize SOA epimers was systematically screened, and acetonitrile was used to dissolve and as the modifier in SFC. Then, key parameters of ultra-high performance SFC (ultra-performance convergence chromatography, UPC²), comprising stationary phase, additive in modifier, column temperature, ABPR pressure, and flow rate, were optimized in sequence. Two isocratic UPC² methods were developed on the achiral Torus 1-AA and Torus Diol columns, suitable for UV and MS detection, respectively. MCI gel column chromatography fractionated the U. macrophylla extract into two mixtures (R/IR and C/CB). Preparative SFC, using a Viridis Prep Silica 2-EP OBD column and acetonitrile-0.2% diethylamine in CO₂ as the mobile phase, was finally employed for compound purification. As a result, the purity of four SOA compounds was all higher than 95%. Different from reversed-phase HPLC, SFC, by use of water-free mobile phase (inert CO₂ and aprotic modifier), provides a solution to rapid analysis and isolation of tautomeric reference standards for quality control of TCM.

http://dx.doi.org/10.1016/j.jpba.2016.10.021
Evaluation Of Supercritical Fluid Chromatography For Testing Of PEG Adducts In Pharmaceuticals

Keywords: University of Copenhagen, Waters, cetirizine, indomethacin, polyethylene glycol, drug-excipient, pharmaceutical

Abstract
Drug formulations containing polyethylene glycol may give rise to formation of reaction products between the aforementioned and the active pharmaceutical ingredient. Supercritical fluid chromatography has recently achieved new interest and improved instrumentation is now available. Here, supercritical fluid chromatography has been evaluated for its possible use for determination of reactions products formed between polyethylene glycol and active pharmaceutical ingredients. A mixture of polyethylene glycols with average molecular weights of 400-6000Da was separated with supercritical fluid chromatography using silica columns and carbon dioxide modified with methanol as mobile phase. Satisfactory resolution (Rs=1.2) of the individual oligomers up to a molecular weight of 1000Da was obtained using evaporative light scattering as detection technique. The active pharmaceutical ingredients, cetirizine or indomethacin were investigated in a reaction mixture containing polyethylene glycol 400 after incubation at 80°C for 120h. Polyethylene glycol esters formed upon reaction with both active pharmaceutical ingredients were observed as polymeric patterns with ultraviolet detection and identified with mass spectrometry. Cetirizine was observed to be more reactive than indomethacin. The observed difference in reactivity is due to differences in polar and steric effects between cetirizine and indomethacin. Evaporative light scattering, ultraviolet absorbance and mass spectrometric detection were investigated and each detection technique has its own advantages and disadvantages, but in order to be able to detect selected impurities in the complex mixture of impurities formed, mass spectrometry is superior.

http://dx.doi.org/10.1016/j.jpba.2013.08.039
167. Simultaneous Quantitation Of The Diastereoisomers Of Scholarisine And 19-Epischolarisine, Vallesamine And Picrine In Rat Plasma By Supercritical Fluid Chromatography With Tandem Mass Spectrometry And Its Application To A Pharmacokinetic Study

Keywords: Jilin University, Waters, pharmaceutical

Abstract
Dengtaiye tablet has been used to treat chronic bronchitis cough. Scholarisine, 19-epischolarisine, vallesamine, and picrine are the representative constituents of Dengtaiye. A rapid and sensitive assay based on supercritical fluid chromatography with tandem mass spectrometry has been developed and validated for the determination of the diastereoisomers of scholarisine and 19-epischolarisine, vallesamine, and picrine in rat plasma using lamotrigine as internal standard. The analysis in a run time of only 6 min was performed on an ACQUITY UPC² Trefoil™ BEH 2-EP column (3.0 x 150 mm, 2.5 μm) at 50°C. The mobile phase consisting of carbon dioxide and methanol (2 mM ammonium formate) was performed as follows: 15% methanol (2 mM ammonium formate) maintained at 0–2 min, 15–19% methanol (2 mM ammonium formate) at 2–4 min, 19–15% methanol (2 mM ammonium formate) at 4–6 min. The flow rate was 1.50 mL/min. The assay was linear over the concentration ranges 50–10000 pg/mL for scholarisine, 19-epischolarisine, vallesamine, and picrine with corresponding lower limits of quantitation of 50 pg/mL. Intra- and interday precisions were in the range 1.42–12.85% with accuracies in the range –11.71–2.48%. The method was successfully applied to a pharmacokinetic study involving a single oral administration of 108 mg/kg Dengtaiye tablet to rats.

http://dx.doi.org/10.1002/jssc.201600243

168. Simultaneous Determination Of 16 Polycyclic Aromatic Hydrocarbons In Reclaimed Water Using Solid-Phase Extraction Followed By Ultra-Performance Convergence Chromatography With Photodiode Array Detection

Keywords: Beijing University of Chemical Technology, Beijing Agro-Monitoring Station, environmental

Abstract
A new fast and effective analysis method has been developed to simultaneously determine 16 polycyclic aromatic hydrocarbons in reclaimed water samples by ultra-performance convergence chromatography with photodiode array detection and solid-phase extraction. The parameters of ultra-performance convergence chromatography on the separation behaviors and the crucial condition of solid-phase extraction were investigated systematically. Under optimal conditions, the 16 polycyclic aromatic hydrocarbons could be separated within 4 min. The limits of detection and quantification were in the range of 0.4–4 and 1–10 μg/L in water, respectively. This approach has been applied to a real industrial wastewater treatment plant successfully. The results showed that polycyclic aromatic hydrocarbons were dramatically decreased after chemical treatment procedure, and the oxidation procedure was effective to remove trace polycyclic aromatic hydrocarbons.

http://dx.doi.org/10.1002/jssc.201500823

169. Characterization Of The Pigment Fraction In Sweet Bell Peppers (Capsicum Annuum L.) Harvested At Green And Overripe Yellow And Red Stages By Off-Line Multidimensional Convergence Chromatography/Liquid Chromatography-Mass Spectrometry

Keywords: University of Messina, Convergence chromatography, Liquid chromatography, Mass spectrometry, overripe fruits, Pigments, Sweet bell peppers

Abstract:
Off-line multidimensional supercritical fluid chromatography combined with reversed-phase liquid chromatography was employed for the carotenoid and chlorophyll characterization in different sweet bell peppers (Capsicum annuum L.) for the first time. The first dimension consisted of an Acquity HSS C₁₈ SB (100 mm x 3 mm I.D., 1.8 μm particles) column operated with a supercritical...
mobile phase in a ultra performance convergence chromatography system, whereas the second dimension was performed in reversed phase mode with a C30 (250 mm × 4.6 mm I.D., 3.0 μm particles) stationary phase combined with photodiode array and mass spectrometry detection. This approach allowed the determination of 115 different compounds belonging to chlorophylls, free xanthophylls, free carotenes, xanthophyll monoesters and xanthophyll diesters, and proved to be a significant improvement in the pigments determination compared to the conventional one-dimensional liquid chromatography approach so far applied to the carotenoid analysis in the studied species. Moreover, the present study also aimed to investigate and to compare the carotenoid stability and composition in overripe yellow and red bell peppers collected directly from the plant, thus also evaluating whether biochemical changes are linked to carotenoid degradation in the non climacteric investigated fruits, for the first time.

http://dx.doi.org/10.1002/jssc.201600220

170.Development And Validation Of An Ultra-Performance Convergence Chromatography Method For The Quality Control Of Angelica Gigas Nakai

Keywords: Korea Institute of Oriental Medicine, Angelica gigas Nakai, Green chemistry, Supercritical fluid chromatography, Ultra-performance convergence chromatography

Abstract

Ultra-performance convergence chromatography, which integrates the advantages of supercritical fluid chromatography and ultra high performance liquid chromatography technologies, is an environmentally friendly analytical method that uses dramatically reduced amounts of organic solvents. An ultra-performance convergence chromatography method was developed and validated for the quantification of decursinol angelate and decursin in Angelica gigas using a CSH Fluoro-Phenyl column (2.1 mm × 150 mm, 1.7 μm) with a run time of 4 min. The method was developed in improved resolution and a shorter analysis time in comparison to the conventional high-performance liquid chromatography method. This method was validated in terms of linearity, precision, and accuracy. The limits of detection were 0.005 and 0.004 μg/mL for decursinol angelate and decursin, respectively, while the limits of quantitation were 0.014 and 0.012 μg/mL, respectively. The two components showed good regression (correlation coefficient (r²) > 0.999), excellent precision (relative standard deviation < 2.28%), and acceptable recoveries (99.75–102.62%). The proposed method can be used to efficiently separate, characterize, and quantify decursinol angelate and decursin in Angelica gigas and its related medicinal materials or preparations, with the advantages of a shorter analysis time, greater sensitivity, and better environmental compatibility.

http://dx.doi.org/10.1002/jssc.201600275

171.Profiling Adrenal 11β-Hydroxyandrostenedione Metabolites In Prostate Cancer Cells, Tissue And Plasma: UPC2-MS/MS Quantification Of 11β-Hydroxytestosterone, 11keto-Testosterone And 11keto-Dihydrotestosterone

Keywords: Stellenbosch University, prostate cells, prostate cancer, Adrenal C19 steroids, active androgens, health science

Abstract

Adrenal C19 steroids serve as precursors to active androgens in the prostate. Androstenedione (A4), 11β-hydroxyandrostenedione (11OHA4) and 11β-hydroxytestosterone (11OHT) are metabolised to potent androgen receptor (AR) agonists, dihydrotestosterone (DHT), 11-ketotestosterone (11KT) and 11-ketodihydrotestosterone (11KDHT). The identification of 11OHA4 metabolites, 11KT and 11KDHT, as active androgens has placed a new perspective on adrenal C11-oxy C19 steroids and their contribution to prostate cancer (PCa).

We investigated adrenal androgen metabolism in normal epithelial prostate (PNT2) cells and in androgen-dependent prostate cancer (LNCaP) cells. We also analysed steroid profiles in PCa tissue and plasma, determining the presence of the C19 steroids and their derivatives using ultra-performance liquid chromatography (UHPLC)- and ultra-performance convergence chromatography tandem mass spectrometry (UPC2-MS/MS).

In PNT2 cells, sixty percent A4 (60%) was primarily metabolised to 5α-androstanedione (5αDIONE) (40%), testosterone (T) (10%), and androsterone (AST) (10%). T (30%) was primarily metabolised to DHT (10%) while low levels of A4, 5αDIONE and 3αADIOL (≈20%) were...
detected. Conjugated steroids were not detected and downstream products were present at <0.05 μM. Only 20% of 11OHA4 and 11OHT were metabolised with the former yielding 11keto-androstenedione (11KAA4), 11KDHT and 11β-hydroxy-5α-androstane (11OH-5αDIONE) and the latter yielding 11OHA4, 11KT and 11KDHT with downstream products <0.03 μM. In LNCaP cells, A4 (90%) was metabolised to AST-glucuronide via the alternative pathway while T was detected as T-glucuronide with negligible conversion to downstream products. 11OHA4 (80%) and 11OHT (60%) were predominantly metabolised to 11KA4 and 11KT and in both assays more than 50% of 11KT was detected in the unconjugated form. In tissue, we detected C11-oxy C19 metabolites at significantly higher levels than the C19 steroids, with unconjugated 11KDHT, 11KT and 11OHA4 levels ranging between 13 and 37.5 ng/g. Analyses of total steroid levels in plasma showed significant levels of 11OHA4 (≈230–440 nM), 11KT (≈250–390 nM) and 11KDHT (≈19 nM). DHT levels (<0.14 nM) were significantly lower.

In summary, 11β-hydroxysteroid dehydrogenase type 2 activity in PNT2 cells was substantially lower than in LNCaP cells, reflected in the conversion of 11OHA4 and 11OHT. Enzyme substrate preferences suggest that the alternate pathway is dominant in normal prostate cells. Glucuronidation activity was not detected in PNT2 cells and while all T derivatives were efficiently conjugated in LNCaP cells, 11KT was not. Substantial 11KT levels were also detected in both PCa tissue and plasma. 11OHA4 therefore presents a significant androgen precursor and its downstream metabolism to 11KT and 11KDHT as well as its presence in PCa tissue and plasma substantiate the importance of this adrenal androgen.

http://dx.doi.org/10.1016/j.jsbmb.2016.06.009

172. Chiral Analysis Of Poor UV Absorbing Pharmaceuticals By Supercritical Fluid Chromatography-Charged Aerosol Detection

2016 - The journal of Supercritical Fluids

Keywords: Merck, enantiopurity, high-throughput analysis, SFC-CAD, pharmaceuticals

Abstract
Fast and efficient chiral analysis of drugs and synthetic intermediates with weak UV absorption is one of the most challenging analytical tasks in modern pharmaceutical research and development. In this study, the use of supercritical fluid chromatography-charged aerosol detection (SFC-CAD) for chiral analysis of poor UV absorbing analytes commonly encountered during pharmaceutical process research was investigated. Investigation of makeup flow rate showed that CAD responses remained largely steady from 1 to 1.5 mL/min for different mobile phase compositions. The effect of the additives in the mobile phase and the makeup flow on the background noise of CAD detection was also studied. Accurate measurement of the enantiopurity of pharmaceuticals from −100% to 100% enantiomeric excess (ee) was achieved under isocratic conditions. The SFC-CAD system was used as a chromatographic screening system for method development and successfully applied to high-throughput chiral analysis of reaction mixtures in microtiter plates.

http://dx.doi.org/doi:10.1016/j.supflu.2016.04.014
173. Evaluation Of The Migration Of UV-Ink Photoinitiators From Polyethylene Food Packaging By Supercritical Fluid Chromatography Combined With Photodiode Array Detector And Tandem Mass Spectrometry

Keywords: Beijing University of Chemical Technology, Beijing Center for Disease Control and Prevention, Chinese Academy of Inspection and Quarantine, UV-ink photoinitiators, polyethylene food packaging migration, leachables, chemical materials

Abstract
UV-ink photoinitiators (PIs), which are used to initiate polymerization reaction for the curing of inks and lacquers, have become a kind of contaminant residues in printing plastic food packaging. The residual PIs in packaging may pose a potential threat to customers. In this work, migration behaviors of 13 PIs from a polyethylene (PE) packaging to food simulants according to regulation EU No 10/2011 were studied by supercritical fluid chromatography combined with photodiode array detector and tandem mass spectrometry (SFC-PDA-MS/MS). The method simultaneously analyzed 13 PIs within 4.5 min and was sensitively with low limits of detection of 0.02–2.16 μg/L, which could meet high throughput analysis for control the quality of food packaging. The migration results revealed that Irgacure 819, Darocure 1173 and TPO, which had low migration rates, should be preferably selected by plastic food packaging manufacture for food safety.

http://dx.doi.org/10.1016/j.polymertesting.2016.06.008

174. Lipid Profiling Of Polarized Human Monocyte-Derived Macrophages

Keywords: Cytokines, Immunology, Mass spectrometry, Phospholipases, Phospholipids

Abstract
The highly orchestrated transcriptional and metabolic reprogramming during activation drastically transforms the main functions and physiology of human macrophages across the polarization spectrum. Lipids, for example, can modify protein function by acting remotely as signaling molecules but also locally by altering the physical properties of cellular membranes. These changes play key roles in the functions of highly plastic immune cells due to their involvement in inflammation, immune responses, phagocytosis and wound healing processes. We report an analysis of major membrane lipids of distinct phenotypes of resting (M0), classically activated (M1), alternatively activated (M2a) and deactivated (M2c) human monocyte derived macrophages from different donors. Samples were subjected to supercritical fluid chromatography-ion mobility-mass spectrometry analysis, which allowed separations based on lipid class, facilitating the profiling of their fatty acid composition. Different levels of arachidonic acid mobilization as well as other fatty acid changes were observed for different lipid classes in the distinct polarization phenotypes, suggesting the activation of highly orchestrated and specific enzymatic processes in the biosynthesis of lipid signaling molecules and cell membrane remodeling. Thromboxane A2 production appeared to be a specific marker of M1 polarization. These alterations to the global composition of lipid bi-layer membranes in the cell provide a potential methodology for the definition and determination of cellular and tissue activation states.

http://dx.doi.org/10.1016/j.prostaglandins.2016.11.002

175. Tandem Mass Spectrometry Determined Maternal Cortisone To Cortisol Ratio And Psychiatric Morbidity During Pregnancy–Interaction With Birth Weight

Keywords: Upsalla University, University of Groningen, Karolinska Institutet, 11-β-hydroxysteroid dehydrogenase, cortisol, cortisone, Separation of steroids by ultra performance convergence chromatography (UPC² with Xevo TQS), health science

Abstract
Maternal serum cortisol has been suggested to be influenced by psychiatric morbidity, and may also influence fetal growth. However, several studies found equal cortisol levels in depressed and healthy pregnant women. Placental 11-β-hydroxysteroid dehydrogenase type 2 (11B-HSD2) shields the fetus from maternal cortisol by conversion to cortisone, a function that may be compromised by maternal stress. We aimed to compare the serum ratio of cortisone to cortisol, in women with and without psychiatric morbidity during pregnancy. A secondary aim was to investigate whether fetal growth, approximated by infant birth weight, was associated with the cortisone to cortisol ratio. We performed tandem mass spectrometry analysis of serum cortisol and
cortisone in late pregnancy in 94 women with antenatal psychiatric morbidity and 122 controls (cohort 1). We also compared the placental gene expression of HSD11B1 and 2 in another group of 69 women with psychiatric morbidity and 47 controls (cohort 2). There were no group differences in cortisol to cortisone ratio, absolute levels of cortisone and cortisol (cohort 1), or expression of HSD11B1 or 2 (cohort 2). However, cortisone to cortisol ratio was positively associated with birth weight in women with psychiatric morbidity, also after adjustment for gestational length, fetal sex, maternal height, smoking, SSRI use, and time of blood sampling (standardized β=0.35, p<0.001), with no association in the healthy controls. Thus, the maternal serum cortisone to cortisol ratio does not seem to be affected by psychiatric morbidity, but psychiatric morbidity may increase fetal exposure to cortisol or other metabolic factors influencing fetal growth.


176. Identifying The Tobacco Related Free Radicals By UPCC-QTOF-MS With Radical Trapping Method In Mainstream Cigarette Smoke

Keywords: Tongji University, Shanghai, Shanghai Tobacco Group Corporation Limited, Ultra-performance convergence chromatography, Radicals, Cigarette smoke, Spin trapping

Abstract

Tobacco related free radicals (TFRs) in the cigarette smoke are specific classes of hazardous compounds that merit concern. In this study, we developed a hybrid method to identify TFRs directly based on ultra-performance convergence chromatography with a quadrupole time-of-flight mass spectrometry (UPCC-QTOF MS) combined spin trapping technique. The short-lived TFRs were stabilized successfully in situ through spin trapping procedure and UPCC was applied to facilitate efficient separation of complex derivative products. Coupling of orthogonal partial least squares discriminant analysis (OPLS-DA), UPCC-QTOF MS system enabled us to identify specific potential TFRs with exact chemical formula. Moreover, computational stimulations have been carried out to evaluate the optimized stability of TFRs. This work is a successful demonstration for the application of an advanced hyphenated technique for separation of TFRs with short detection time (less than 7 min) and high throughput.

http://dx.doi.org/10.1016/j.talanta.2016.07.002

177. Analysis Of Hydroxylated Polybrominated Diphenyl Ethers (OH-Bdes) By Supercritical Fluid Chromatography/Mass Spectrometry

KeyWords: The State University of New York, Waters Corporation, Hydroxylated Polybrominated Diphenyl Ethers (OH-BDEs), Supercritical Fluid Chromatography (SFC), Mass Spectrometry (MS), Metabolism, Human Serum

Abstract

Hydroxylated polybrominated diphenyl ethers (OH-BDEs), which have anthropogenic and natural origins, have exhibited neurotoxic and endocrine disrupting effects in humans and wildlife. Therefore, there is an increased interest in the analysis of these compounds in biological matrices in order to assess their potential toxicological risks. Analysis of OH-BDEs is conventionally completed using liquid chromatography/mass spectrometry (LC/MS), or by gas chromatography/mass spectrometry (GC/MS) after derivatization. Issues with resolution in separating congeners have limited the analysis of OH-BDEs via LC/MS, with published methods only able to include 13 congeners in the analysis. On the other hand, while GC/MS analysis can analyze more OH-BDE congeners, derivatization of OH-BDEs to convert them to GC amenable compounds adds to sample preparation time and limits the column lifetime due to trace residues of highly reactive derivatization agents entering the column. Herein we report the development of a supercritical fluid chromatography/mass spectrometry (SFC/MS) method for the analysis of 22 OH-BDE congeners. Instrument limits of detection for the developed method ranged from 2 to 106 fg injected on column, which is lower than previously optimized LC/MS and GC/MS methods. The developed SFC/MS method was successfully applied towards the analysis of in vitro metabolism samples and human serum samples to demonstrate its applicability with different biological matrices.

http://dx.doi.org/10.1016/j.talanta.2016.08.013
178. Stereoselective Quantification Of Triticonazole In Vegetables By Supercritical Fluid Chromatography

Keywords: Triticonazole, Enantioseparation, Supercritical fluid chromatography, Influence of organic modifier, Stereoselective quantification, QuEChERS pretreatment

Abstract:
A highly fast analytical method though supercritical fluid chromatography (SFC) has been developed to quantify triticonazole enantiomers in cucumbers and tomatoes. Effects of organic modifier type and concentration on chiral separation and quantification of standard solution as well as matrix-matched standard solutions have been studied in detail. Among three organic modifiers, better separation of triticonazole racemate was achieved with 20% ethanol (v/v). The run time in SFC (ca 3 min) with CO2-ethanol (80:20, v/v) as the mobile phase was six-fold shorter than HPLC analysis (about 18 min). Then, QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction procedure was used for triticonazole in vegetables. The residue analysis method was validated. Good linearity (R2≥0.9988) and recoveries (81.62~106.21%, RSD ≤ 7.30%) for the two enantiomers were achieved. This developed method described herein is convenient and reliable for enantioselective detection of triticonazole in vegetables, which might provide additional information for reliable risk assessment of chiral pesticides.

http://dx.doi.org/10.1016/j.talanta.2016.08.077

179. Ethynylogation Approach In Pharmacophore Design: From Alkynyl-To Butadiynyl-Carbinols Vs Antitumoral Cytotoxicity

Keywords: Alkyne, Asymmetric synthesis, Antitumor agent, Cytotoxicity, Dialkynylcarbinol, 1,3-Diyne, Ethynylogation, Lipidic alkynylcarbinol

Abstract:
Ethynylogation of a chiral lipidic dialkynylcarbinol (DAC), identified as a lead for cytotoxicity against HCT116 cancer cells, is shown to typify the butadiynyl-alkynylcarbinol (BAC) unit as a new pharmacophore. The enantiomers of the internal BAC have been synthesized with 72–75% yield and 85% ee through the use of a modified Carreira reaction shown here for the first time to be compatible with butadiyne and ynal substrates. One enantiomer of the internal BAC could be characterized by X-ray crystallography. In this particular case, the 'DAC to BAC' ethynylogation results in a slight enhancement of the eutomer potency with a preserved vanishing eudismic ratio (IC50 values from 102±14 nM to 42±12 nM for the (+) enantiomers).

http://dx.doi.org/10.1016/j.tet.2016.09.001
180. Pesticides Determination In Fruit Samples Using SFC-DAD

Keywords: Pesticides, UPC2, QuEChERS, DisQue, Papaya, avocado, lime, banana, mango, melon

Abstract:
The development of analytical methods for pesticides determination in fruits is an important task due to the toxicity of these compounds. To improve pesticides analysis in food matrices alternative chromatographic techniques can be used. Supercritical fluid chromatography (SFC) has barely been applied in this area. Atrazine (ATZ), ametryn (AME), carbofuran (CF), carbaryl (CAR) and methyl parathion (MeP), QuEChERS, DisQuE kit, column Viridis BEH 2-EP (100 mm x 4.6 mm, 5um) and SFC–DAD system Acquity UPC2 (Waters). Fruits samples were subjected to QuEChERS AOAC Official Method. For chromatographic analysis, supercritical CO2 and MeOH were used as mobile phase. The MeOH gradient was: 5% for 0.4 min, from 5 to 60% in 1.6 min and 60% for 3 min. Pressure, flow rate and column temperature was 1500 psi, 1.5 mL/min and 40 °C, respectively. MeP was detected at 265 nm and the others at 218 nm. Pesticides were separated in 2.2 min with a resolution >1.5. The method was validated using papaya and avocado samples. From selectivity assess, interferences were no observed for the studied pesticides in both fruits, except for CF. For papaya LODs and LOQs ranged from 0.13–0.38 and 0.22–0.64 g/g, respectively; recoveries varied from 72.8–94.6%; RSD was <3%; and the correlation coefficient (r) values were >0.99. While for avocado LODs and LOQs ranged from 0.12–0.45 and 0.28–0.80 g/g; respectively; RSD was <11%; recoveries varied from 50.0 to 94.2%; and the r values were >0.98. Matrix effect determined in both fruits was considered medium signal suppression. The method applicability was assessed for lime, banana, mango and melon samples. It was observed that this method can be used for the analysis of MeP and ATZ in the four fruits and for CAR and AME in mango and melon. A SFC-DAD method for pesticides analysis in papaya and avocado was developed. This method showed a good performance and satisfactory validation parameters. This method proved to be useful for other fruits.

http://dx.doi.org/10.1016/j.toxlet.2016.07.222

181. The Emergence Of Low-Cost Compact Mass Spectrometry Detectors For Chromatographic Analysis

Keywords: Merck, Method development; High-throughput analysis; Pharmaceutical analysis; Process chemistry support

Abstract:
An overview of recent progress in the development of compact mass spectrometers for use as chromatographic detectors in chemical analysis is presented. As the applications of LC-MS technologies have grown in recent years there has been a continued expansion of the approach to new user groups. Within the pharmaceutical industry, the recent development of small, inexpensive and quiet MS detectors for LC has enabled the rollout of this important technology well beyond the initial user base of researchers in drug metabolism and bioanalysis to the direct support of research areas such as discovery chemistry, process chemistry, chemical engineering, manufacturing and formulation sciences, with comparable broadening of the MS user base occurring in other industries and in academia. In this review we survey recent developments and applications ranging from reaction monitoring, biomolecule analysis and high-throughput microplate analysis to the identification and analysis of impurities, degradation products and potential mutagens, offering thoughts on current limitations and future directions.
182. Metamorphosis Of Supercritical Fluid Chromatography To SFC – An Overview

Keywords: Waters, Tarafder, Method; Stationary phase; Application; Metabolite; Pharmaceutical; Forensic; Food

Abstract
SFC is expanded as supercritical fluid chromatography, but the chromatography that is currently conducted under the name of SFC are not dependent on supercritical conditions. An SFC practitioner is mostly neither aware of the critical temperatures and pressures of the solvents, nor even require to take any measures to maintain supercriticality. Because according to the current author, SFC has decisively shifted its focus from using mobile phase in supercritical conditions to using carbon dioxide as mobile phase, mixed with other co-solvents. SFC has discovered the benefits of using compressed CO₂ as solvent, which opened up some distinctly different and significantly wide application possibilities for chromatographic analyses as a whole. Here an overview will be provided on how supercritical fluid chromatography metamorphosed to SFC and why SFC may no longer be considered as a technique with some special application scope, rather a complementary tool to some widely accepted analytical techniques e.g. reversed-phase chromatography.

doi:10.1016/j.trac.2016.01.002

183. Supercritical Fluids – The Current State And Outlook

Keywords: RESEARCH ON THE APPLICATION OF SUPERCRITICAL FLUIDS, EXTRACTION, FRACTIONATION, FOAMS, NANOPARTICLES

Summary
The main results of the research in the Laboratory of Supercritical Fluids of the Institute of Chemical Process Fundamentals in Prague involve mathematical models for the kinetics of supercritical fluid extraction from plants, distinguished according to the plants and extracted substances, preparations based on CO₂ extracts for the application as botanical insecticides, and enzymatic reactions of oil in supercritical CO₂ medium. The contemporary research is focused on the online fractionation of CO₂ extracts, pressurized liquid extraction, CO₂ assisted foaming of polymers, and recrystallization of the films of metal oxides in watermodified supercritical CO₂.
184. Direct Analysis Of Lipophilic Antioxidants Of Olive Oils Using Bicontinuous Microemulsions

Keywords: Olive oil phenolics, lipophilic antioxidants, antioxidant capacity of EVOO

Abstract
Quantitative analyses of olive oil for lipophilic antioxidants, such as α-tocopherol and phenolics, by simple electrochemical measurements were conducted in a bicontinuous microemulsion (BME), which was bicontinuously composed of saline and toluene microphases with a surfactant system. Lipophilic antioxidants in oils were directly monitored in BME solutions using a lipophilic, fluorinated nanocarbon-film electrode (F–ECR). The combination of a well-balanced BME and extremely biased electrodes, such as strongly hydrophilic indium/tin oxide and strongly lipophilic (hydrophobic) F–ECR, allowed individual monitoring of hydrophilic and lipophilic antioxidants in the same BME solution without any required extraction. Furthermore, values for the charge $Q$, integrated from observed currents, showed good linear relationships with the results of conventional assays for antioxidant activity, namely, total phenolics and oxygen radical absorbance capacity assays, even with practical food samples. This proposed methodology provided a very simple, rapid, easily serviceable, and highly reproducible analysis that possesses great potential for applications to a wide range of chemical mixtures, in terms of analyte and media, beyond food oils.

http://dx.doi.org/10.1021/acs.analchem.5b03445

185. Enantioselective Simultaneous Analysis Of Selected Pharmaceuticals In Environmental Samples By Ultrahigh Performance Supercritical Fluid Based Chromatography Tandem Mass Spectrometry

Keywords: University of Bath, Norwegian Institute for Water Research, NIVA, chiral pharmaceutically active compounds, influent, effluent, amylose column, cellulose column, environmental

Abstract
In order to assess the true impact of each single enantiomer of pharmacologically active compounds (PACs) in the environment, highly efficient, fast and sensitive analytical methods are needed. For the first time this paper focuses on the use of ultrahigh performance supercritical fluid based chromatography coupled to a triple quadrupole mass spectrometer to develop multi-residue enantioselective methods for chiral PACs in environmental matrices. This technique exploits the advantages of supercritical fluid chromatography, ultrahigh performance liquid chromatography
and mass spectrometry. Two coated modified 2.5 μm-polysaccharide-based chiral stationary phases were investigated: an amylose tris-3,5-dimethylphenylcarbamate column and a cellulose tris-3-chloro-4-methylphenylcarbamate column. The effect of different chromatographic variables on chiral recognition is highlighted. This novel approach resulted in the baseline resolution of 13 enantiomers PACs (aminorex, carprofen, chloramphenicol, 3-N-dechloroethylifosfamide, flurbiprofen, 2-hydroxyibuprofen, ifosfamide, imazalil, naproxen, ofloxacin, omeprazole, praziquantel and tetramisole) and partial resolution of 2 enantiomers PACs (ibuprofen and indoprofen) under fast-gradient conditions (<10 min analysis time). The overall performance of the methods was satisfactory. The applicability of the methods was tested on influent and effluent wastewater samples. To the best of our knowledge, this is the first feasibility study on the simultaneous separation of chemically diverse chiral PACs in environmental matrices using ultrahigh performance supercritical fluid based chromatography coupled with tandem mass spectrometry.

http://dx.doi.org/10.1016/j.aca.2016.05.051

186. Development And Optimization Of Ultra-High Performance Supercritical Fluid Chromatography Mass Spectrometry Method For High-Throughput Determination Of Tocopherols And Tocotrienols In Human Serum

Keywords: Charles University, Palacky University, Research Institute of Brewing and Malting, vitamin E, pharmaceutical

Abstract
The goal of this study was to develop an effective supercritical fluid chromatography method using single quadrupole MS for analysis of all isomeric forms of vitamin E. Finally, two fast and effective methods, the high resolution one and the high speed one, for the determination of 8 vitamin E isomers in human serum were developed.

Rapid high-throughput liquid-liquid extraction was selected as a sample preparation step. Sample pretreatment of 100 μL human serum was consisted of protein precipitation with 200 μL ethanol and liquid-liquid extraction by 400 μL hexane/dichloromethane (80/20, v/v). The separation was performed on BEH 2-EP (3.0 × 100 mm, 1.7 μm) stationary phase, using isocratic elution with carbon dioxide and 10 mM ammonium formate in methanol in the ratio 98:2 for high resolution method with run time 4.5 min and in the ratio 95:5 for high speed method, where the run time was 2.5 min. The method development included optimization of key parameters: the choice of the suitable stationary phase and the composition of mobile phase, where an influence of various modifiers, their ratio and additives were tested, and optimization of fine tuning parameters including BPR pressure, flow-rate and column temperature. Quantification of all isomeric forms was performed using SIM (single ion monitoring) experiments in ESI positive ion mode. Both high speed and high resolution chromatographic methods were validated in terms of precision, accuracy, range, linearity, LOD, LOQ and matrix effects using the same LLE procedure. The high resolution method provided more sensitive results (LOD: 0.017–0.083 μg mL⁻¹) and better linearity (r² > 0.9930) than the high speed one (LOD: 0.083–0.25 μg mL⁻¹, r² > 0.9877) at the cost of double time of analysis.

http://dx.doi.org/10.1016/j.aca.2016.06.008
187. Simultaneous Enantioselective Determination Of Triadimefon And Its Metabolite Triadimenol In Edible Vegetable Oil By Gel Permeation Chromatography And Ultraprecision Convergence Chromatography / Tandem Mass Spectrometry

Keywords: Zhejiang University, Shanghai Institute of Organic Chemistry, stereoisometric separation, triadimenol, triadimefones, , vegetable oil, food

Abstract
A novel, sensitive, and efficient enantioselective method for the determination of triadimefon and its metabolite triadimenol in edible vegetable oil, was developed by gel permeation chromatography and ultraperformance convergence chromatography/tandem triple quadrupole mass spectrometry. After the vegetable oil samples were prepared using gel permeation chromatography, the eluent was collected, evaporated, and dried with nitrogen gas. The residue was redissolved by adding methanol up to a final volume of 1 mL. The analytes of six enantiomers were analyzed on Chiralpak IA-3 column (150 × 4.6 mm) using compressed liquid CO2-mixed 14 % co-solvents, comprising ethanol / acetonitrile / isopropanol =20/20/60 (v/v/v) in the mobile phase at 30 °C, and the total separation time was less than 4 min at a flow rate of 2 mL/min. Quantification was achieved using matrix-matched standard calibration curves. The overall mean recoveries for six enantiomers from vegetable oil were 90.1–97.3 %, with relative standard deviations of 0.8–5.4 % intra-day and 2.3–5.0 % inter-day at 0.5, 5, and 50 μg/kg levels. The limits of quantification were 0.5 μg/kg for all enantiomers based on five replicate extractions at the lowest fortified level in vegetable oil. Moreover, the absolute configuration of six enantiomers had been determined based on comparisons of the vibrational circular dichroism experimental spectra with the theoretical curve obtained by density functional theory calculations. Application of the proposed method to the 40 authentic vegetable oil samples from local markets suggests its potential use in enantioselective determination of triadimefon and triadimenol enantiomers.


188. Analytical Method Development For The Determination Of Emerging Contaminants In Water Using Super-Critical Fluid Chromatography Coupled With Diode-Array Detection

Keywords: Universidad de Colima, emerging contaminants, Viridis BEH 2-EP, water samples, endocrine distruptors, triclosan, diuran, glyburide, carbamazepine, estradiol, ethinyl estradiol, bisphenol A, environmental

Abstract
An analytical method using supercritical-fluid chromatography coupled with diode-array detection for the determination of seven emerging contaminants—two pharmaceuticals (carbamazepine and glyburide), three endocrine disruptors (17α-ethinyl estradiol, bisphenol A, and 17β-estradiol), one bactericide (triclosan), and one pesticide (diuron)—was developed and validated. These contaminants were chosen because of their frequency of use and their toxic effects on both humans and the environment. The optimized chromatographic separation on a Viridis BEH 2-EP column achieved baseline resolution for all compounds in less than 10 min. This separation was applied to environmental water samples after sample preparation. The optimized sample treatment involved a preconcentration step by means of solid-phase extraction using C18-OH cartridges. The proposed method was validated, finding recoveries higher than 94 % and limits of detection and limits of quantification in the range of 0.10–1.59 μg L⁻¹ and 0.31–4.83 μg L⁻¹, respectively. Method validation established the proposed method to be selective, linear, accurate, and precise. Finally, the method was successfully applied to environmental water samples.

http://dx.doi.org/10.1007/s00216-015-8581-x

189. Analysis Of Glucuronide And Sulfate Steroids In Urine By Ultra-High-Performance Supercritical-Fluid Chromatography Hyphenated Tandem Mass Spectrometry

Keywords: LUNAM University, LABERCA, steroid profiling, conjugated steroids, glucuronide, sulfate steroids, anabolic steroids, steroid isomers, hormones, veterinary drug residue, bovine urine, food
Profiling conjugated urinary steroids to detect anabolic-steroid misuse is recognized as an efficient analytical strategy in both chemical-food-safety and anti-doping fields. The relevance and robustness of such profiling rely on the analysis of glucuronide and sulfate steroids, which is expected to have properties including accuracy, specificity, sensitivity, and, if possible, rapidity. In this context, the ability of ultra-high-performance supercritical-fluid chromatography (UHPSFC) hyphenated tandem mass spectrometry (MS–MS) to provide reliable and accurate phase II analysis of steroids was assessed. Four stationary phases with sub-2 μm particles (BEH, BEH 2-ethyl-pyridine, HSS C18 SB, and CSH fluorophenyl) were screened for their capacity to separate several conjugated steroid isomers. Analytical conditions including stationary phase, modifier composition and percentage, back pressure, column temperature, and composition and flow rate of make-up solvent were investigated to improve the separation and/or the sensitivity. Thus, an analytical procedure enabling the analysis of eight glucuronide and 12 sulfate steroids by two different methods in 12 and 15 min, respectively, was optimized. The two procedures were evaluated, and UHPSFC–MS–MS analysis revealed its ability to provide sensitive (limits of quantification: 0.1 ng mL\(^{-1}\) and 0.5 ng mL\(^{-1}\) for sulfate and glucuronide steroids, respectively) and reliable quantitative performance (\(R^2 > 0.995\), RSD < 20 %, and bias < 30 %) through the use of suitable labeled internal standards. Comparison with UHPLC–MS–MS was performed, and UHPSFC–MS–MS obtained better performance in terms of sensitivity. Finally, as a proof of concept, this so-called green technology was used in a chemical-food-safety context to profile steroid conjugates in urine samples from bovines treated with estradiol.

http://dx.doi.org/10.1007/s00216-015-8573-x

190. High-Throughput And Comprehensive Lipidomic Analysis Using Ultrahigh-Performance Supercritical Fluid Chromatography–Mass Spectrometry

Keywords: Lipid class separations, ESI-MS, non-polar and polar lipid classes, fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterols, and prenols, biological matrices

New analytical approach for high-throughput and comprehensive lipidomic analysis of biological samples using ultrahigh-performance supercritical fluid chromatography (UHPSFC) with electrospray ionization-mass spectrometry (ESI-MS) is presented in this work as an alternative approach to established shotgun MS or high-performance liquid chromatography-MS. The lipid class separation is performed by UHPSFC method based on 1.7 μm particle-bridged ethylene hybrid silica column with a gradient of methanol–water–ammonium acetate mixture as a modifier. All parameters of UHPSFC conditions are carefully optimized and their influence on the chromatographic behavior of lipids is discussed. The final UHPSFC/ESI-MS method enables a fast separation of 30 nonpolar and polar lipid classes within 6 min analysis covering 6 main lipid categories including fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterols, and prenols. Individual lipid species within lipid classes are identified based on positive and negative-ion full-scan and tandem mass spectra measured with high mass accuracy and high resolving power. Developed UHPSFC/ESI-MS method is applied for the analysis of porcine brain extract as a complex lipidomic sample, where 24 lipid classes containing 436 lipid species are identified. The method is validated for the quantitative analysis of lipid species in biological tissues using internal standards for each lipid class. This high-throughput, comprehensive and accurate UHPSFC/ESI-MS method is suitable for the lipidomic analysis of large sample sets in the clinical research.

http://dx.doi.org/10.1021/acs.analchem.5b01054
Keywords: Charles University, red dye, sudan red, spices, CSH, food

Abstract
A novel simple, fast and efficient ultra-high performance supercritical fluid chromatography (UHPSFC) method was developed and validated for the separation and quantitative determination of eleven illegal dyes in chili-containing spices. The method involved a simple ultrasound-assisted liquid extraction of illegal compounds with tetrahydrofuran. The separation was performed using a supercritical fluid chromatography system and CSH Fluoro-Phenyl stationary phase at 70 °C. The mobile phase was carbon dioxide and the mixture of methanol:acetonitrile (1:1, v/v) with 2.5% formic acid as an additive at the flow rate 2.0 mL min⁻¹. The UV–vis detection was accomplished at 500 nm for seven compounds and at 420 nm for Sudan Orange G, Butter Yellow, Fast Garnet GBC and Methyl Red due to their maximum of absorbance. All eleven compounds were separated in less than 5 min. The method was successfully validated and applied using three commercial samples of chili-containing spices – Chili sauce (Indonesia), Feferony sauce (Slovakia) and Mojo sauce (Spain). The linearity range of proposed method was 0.50–9.09 mg kg⁻¹ (r ≥ 0.995). The detection limits were determined as signal to noise ratio of 3 and were ranged from 0.15 mg kg⁻¹ to 0.60 mg kg⁻¹ (1.80 mg kg⁻¹ for Fast Garnet) for standard solution and from 0.25 mg kg⁻¹ to 1.00 mg kg⁻¹ (2.50 mg kg⁻¹ for Fast Garnet, 1.50 mg kg⁻¹ for Sudan Red 7B) for chili-containing samples. The recovery values were in the range of 73.5–107.2% and relative standard deviation ranging from 0.1% to 8.2% for within-day precision and from 0.5% to 8.8% for between-day precision. The method showed potential for being used to monitor forbidden dyes in food constituents. The developed UHPSFC method was compared to the UHPLC-UV method. The orthogonality of Sudan dyes separation by these two methods was demonstrated. Benefits and drawbacks were discussed showing the reliability of both methods for monitoring of studied illegal dyes in real food constituents.

http://dx.doi.org/10.1016/j.aca.2015.03.003
192. Ultra High Performance Supercritical Fluid Chromatography Coupled With Tandem Mass Spectrometry For Screening Of Doping Agents. I: Investigation Of Mobile Phase And MS Conditions

2015 - Analytica Chimica Acta

Keywords: Doping agents; Make-up solvent; Mobile phase; Ultra high performance liquid chromatography; Ultra high performance supercritical fluid chromatography

Abstract
The conditions for the analysis of selected doping substances by UHPSFC-MS/MS were optimized to ensure suitable peak shapes and maximized MS responses. A representative mixture of 31 acidic and basic doping agents was analyzed, in both ESI+ and ESI- modes. The best compromise for all compounds in terms of MS sensitivity and chromatographic performance was obtained when adding 2% water and 10mM ammonium formate in the CO2/MeOH mobile phase. Beside mobile phase, the nature of the make-up solvent added for interfacing UHPSFC with MS was also evaluated. Ethanol was found to be the best candidate as it was able to compensate for the negative effect of 2% water addition in ESI- mode and provided a suitable MS response for all doping agents. Sensitivity of the optimized UHPSFC-MS/MS method was finally assessed and compared to the results obtained in conventional UHPLC-MS/MS. Sensitivity was improved by 5-100-fold in UHPSFC-MS/MS vs. UHPLC-MS/MS for 56% of compounds, while only one compound (bumetanide) offered a significantly higher MS response (4-fold) under UHPLC-MS/MS conditions. In the second paper of this series, the optimal conditions for UHPSFC-MS/MS analysis will be employed to screen >100 doping agents in urine matrix and results will be compared to those obtained by conventional UHPLC-MS/MS


193. Ultra High Performance Supercritical Fluid Chromatography Coupled With Tandem Mass Spectrometry For Screening Of Doping Agents. II: Analysis Of Biological Samples

2015 - Analytica Chimica Acta
Abstract
The potential and applicability of UHPSFC–MS/MS for anti-doping screening in urine samples were tested for the first time. For this purpose, a group of 110 doping agents with diverse physicochemical properties was analyzed using two separation techniques, namely UHPLC–MS/MS and UHPSFC–MS/MS in both ESI+ and ESI– modes. The two approaches were compared in terms of selectivity, sensitivity, linearity and matrix effects. As expected, very diverse retentions and selectivities were obtained in UHPLC and UHPSFC, proving a good complementarity of these analytical strategies. In both conditions, acceptable peak shapes and MS detection capabilities were obtained within 7 min analysis time, enabling the application of these two methods for screening purposes. Method sensitivity was found comparable for 46% of tested compounds, while higher sensitivity was observed for 21% of tested compounds in UHPLC–MS/MS and for 32% in UHPSFC–MS/MS. The latter demonstrated a lower susceptibility to matrix effects, which were mostly observed as signal suppression. In the case of UHPLC–MS/MS, more serious matrix effects were observed, leading typically to signal enhancement and the matrix effect was also concentration dependent, i.e., more significant matrix effects occurred at the lowest concentrations.

http://dx.doi.org/10.1016/j.aca.2014.10.007


Abstract
Analysis of the brominated flame retardant hexabromocyclododecane (HBCDD) is characterized by the separation of its three predominant diastereomers. This analysis is typically performed using reversed phase liquid chromatography (RPLC) coupled with mass spectrometric (MS) detection with analysis times in the order of 10 minutes or greater. Here we describe a rapid method using supercritical CO2 and methanol to baseline separate the three most abundant HBCDD diastereomers within a three minute run time using a High Strength Silica (HSS) C18 1.8 μm particle size column. A unique elution order of the α-, β- and γ-HBCDD diastereomers using supercritical CO2 was observed, and can be used as an orthogonal separation for further confidence in diastereomer identification when used in conjunction with RPLC. A tandem quadrupole mass spectrometer with negative mode electrospray ionization was used for detection, operating in multiple reaction monitoring (MRM) mode. Ionization was enhanced by the addition of a make-up flow, which was introduced to the post-column effluent. Method limit of detection (LOD) and limit of quantification (LOQ) for α-, β- and γ-HBCDD were based on peak-to-peak signal to noise ratios of greater than 3 or 10, respectively. The LOD for all HBCDD diastereomers as solvent standards was 100 fg on-column, and LOQs 500 fg on-column for α- and γ-HBCDD and 250 fg on-
column for β-HBCDD. In order to test the efficiency of this method, small subsets of complex human serum and whale blubber extracts were analyzed using this method, resulting in positive detections in samples of α-HBCDD.
http://dx.doi.org/10.1039/C4AY02923B

195. Lipidomics: Novel Insight Into The Biochemical Mechanism Of Lipid Metabolism And Dysregulation-Associated Disease

Keywords: Northwest University, State Food and Drug Administration, lipid metabolism, lipids, lipidomics, biomarkers, health science

Abstract
The application of lipidomics, after genomics, proteomics and metabolomics, offered largely opportunities to illuminate the entire spectrum of lipidome based on a quantitative or semi-quantitative level in a biological system. When combined with advances in proteomics and metabolomics high-throughput platforms, lipidomics provided the opportunity for analyzing the unique roles of specific lipids in complex cellular processes. Abnormal lipid metabolism was demonstrated to be greatly implicated in many human lifestyle-related diseases. In this review, we focused on lipidomic applications in brain injury disease, cancer, metabolic disease, cardiovascular disease, respiratory disease and infectious disease to discover disease biomarkers and illustrate biochemical metabolic pathways. We also discussed the analytical techniques, future perspectives and potential problems of lipidomic applications. The application of lipidomics in disease biomarker discovery provides the opportunity for gaining novel insights into biochemical mechanism.
http://dx.doi.org/10.1016/j.cbi.2015.09.005

196. Determination Of 11 Fat Soluble Vitamins (A, D, E, K) And Their Derivatives In Vitamin Tablets By Ultra Performance Convergence Chromatography

Keywords: Ultra performance convergence chromatography; Vitamin tablets; Stereoisomers; Chiral separation

Abstract
A new method was developed for the determination of 11 fat soluble vitamins (A, D, E and K) and its derivatives in vitamin tablets by ultra performance convergence chromatography (UPC²). The mobile phase was the mixture of supercritical CO2 and acetonitrile at a flow rate of 1 mL/min. The separation was carried out on the Waters Acquity UPC² HSS C18 SB 100 mm × 3.0 mm i.d., 1.8 μm column. The UV detector was set at a wavelength of 284 nm. The limits of detection (LOD) were 1.5 - 2.0 mg/L, and the calibration linear for VK1, VK2, VK3 and VB3 was 3-300 mg/L, linear for VA, VA palmitate, VA formic acid, VE, VE acetate, VD2 and VD3 was 5-300 mg/L, respectively. Its spiked recoveries were 97.31% - 98.76%, and the relative standard deviations (RSD’s) were 0.41% - 0.96%. The method is applicable for the determination of fat soluble vitamins (A, D, E and K) and their derivatives in vitamin tablets.
197. Determination Of Bifenthrin In Tea By Ultra Performance Convergence Chromatography And Gas Chromatography-Mass Spectrometry

Keywords: Ultra performance convergence chromatography; Bifenthrin; Tea, GC-MS

Abstract
A method was developed for the determination of bifenthrin in tea by ultra performance convergence chromatography (UPC²). The samples were extracted with petroleum ether and cleaned up with Waters Sep-Pak® Carbon NH₂, and then detected by UPC². The mobile phase was the mixture of supercritical CO₂ and acetonitrile at a flow rate of 1.5 mL/min. The separation was conducted on a column of ACQUITY UPC² BEH (100 mm×3.0 mm,1.7 μm). The UV detector was set at a wavelength of 220 nm. The detection limit was 20 μg/L. The linear range of bifenthrin was 0.32-10.30 mg/L. The recoveries ranged from 88.7% to 98.2%. The relative standard deviations (RSD) were from 1.4% to 2.8%. The result showed that the UPC² was more efficient, rapid and low-cost than GC-MS. The method can meet the testing requirements of bifenthrin in tea. The efficacy of UPC² on testing the concentration of bifenthrin in tea was compared with GC-MS based on National Proficiency Testing and the results were satisfactory.


198. Rapid Detection Of Five Common Fatty Acids In Industrial Oleic Acid Based On Ultra Performance Convergence Chromatography - Mass Spectrometry

Keywords: Ultra performance convergence chromatography; fatty acids; oleic acid, fruit matrix

Abstract
A rapid method was developed for the determination of 5 common fatty acids, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid in industrial oleic acid based on ultra performance convergence chromatography-mass spectrometry ( UPC_MS) . The sample was dissolved by n-hexane, followed by clean_up of extract using 0.22 μm organic phase filter. The fatty acids were separated in 3 min on the column of Acquity UPC2 BEH 2_EP by gradient elution with carbon dioxide and methanol/acetonitrile (1:1, V/V) system, and finally detected by MS detector in ESI- mode. Through the optimization of UPC2_MS condition, the reasonable linearity was achieved for all the analytes over the range of 0.5-100 mg/L with the correlation coefficients (R²) greater than 0. 9985. The recoveries for five fatty acids at three spiked levels were in the range from 89.3% to 106.67% with relative standard deviations of 0.8%-3.0%. The limits of detection for target compounds in the method ranged from 0.07 mg/L to 0.26 mg/L. The real sample analysis showed that this method was simple, fast and had a good separation effect. There was no need of derivatization for fatty acid samples. This work would provide a fast and effective detection method for UPC2 technology in oil related research field.


199. A Silica-Supported Solid Dispersion Of Bifendate Using Supercritical Carbon Dioxide Method With Enhanced Dissolution Rate And Oral Bioavailability

Keywords: Shenyang Pharmaceutical University, bifendate, bioavailability, pharmacokinetics, veterinary, pharmaceuticals

Abstract
In this study, to enhance the dissolution rate and oral bioavailability of bifendate, a silica-supported solid dispersion (SD) of bifendate was prepared using supercritical carbon dioxide (ScCO₂) technology. The properties of bifendate-silica SD were characterized by differential scanning calorimetry (DSC), X-ray diffraction (X-RD) and scanning electron microscopy. The pharmacokinetic study was carried out in beagle dogs using commercial bifendate dropping pills as a reference which is a conventional SD formulation of bifendate and PEG6000. A novel method of Ultra Performance Convergence Chromatography-tandem mass spectrometry (UPC²™-MS/MS) method was applied to determine bifendate concentration in plasma. The amorphous state of bifendate in bifendate-silica SD was revealed in X-RD and DSC when the ratios of bifendate and silica were 1:1.15 and 1:1.19, respectively. In vitro dissolution rate was significantly improved with cumulative release of 67% within 20 min relative to 8% for the physical mixture of bifendate and silica, and which was also higher than the commercial dropping pill of 52%. After storage at 75%
relative humidity (RH) for 10 d, no recrystallization was found and reduced dissolution rate was obtained due to the absorption of moisture. In pharmacokinetic study, C\text{max} and AUC\text{0–t} for bifendate-silica SD were 153.1 ng/ml and 979.8 ng h/ml, respectively. AUC\text{0–t} of bifendate-silica SDs was ∼1.6-fold higher than that of the commercial dropping pills. These results suggest that adsorbing bifendate onto porous silica via ScCO\textsubscript{2} technique could be a feasible method to enhance oral bioavailability together with a higher dissolution rate.

http://www.tandfonline.com/doi/abs/10.3109/03639045.2015.1071833#.VcDcwvlVhBc

200. Determination Of A Selection Of Synthetic Cannabinoids And Metabolites In Urine By UHPSFC-MS/MS And By UHPLC-MS/MS

Keywords: Norwegian Institute of Public Health, synthetic cannabinoids, metabolites in urine, forensic toxicology

Abstract
Two different analytical techniques, ultra-high performance supercritical fluid chromatography–tandem mass spectrometry (UHPSFC-MS/MS) and reversed phase ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS), were used for the determination of two synthetic cannabinoids and eleven metabolites in urine; AM-2201 N-4-OH-pentyl, AM-2233, JWH-018 N-5-OH-pentyl, JWH-018 N-pentanoic acid, JWH-073 N-4-OH-butyl, JWH-073 N-pentanoic acid, JWH-122 N-5-OH-pentyl, MAM-2201, MAM-2201 N-4-OH-pentyl, RCS-4 N-5-OH-pentyl, UR-144 degradant N-pentanoic acid, UR-144 N-4-OH-pentyl, and UR-144 N-pentanoic acid. Sample preparation included a liquid-liquid extraction after deconjugation with β-glucuronidase. The UHPSFC-MS/MS method used an Acquity UPC\textsubscript{2} BEH column with a mobile phase consisting of CO\textsubscript{2} and 0.3% ammonia in methanol, while the UHPLC-MS/MS method used an Acquity UPLC® BEH C\textsubscript{18} column with a mobile phase consisting of 5 mM ammonium formate (pH 10.2) and methanol. MS/MS detection was performed with positive electrospray ionization and two multiple reaction monitoring transitions. Deuterated internal standards were used for six of the compounds. Limits of quantification (LOQs) were between 0.04 and 0.4 µg/L. Between-day relative standard deviations at concentrations ≥ LOQ were ≤20%, with biases within ±19%. Recoveries ranged from 40 to 90%. Corrected matrix effects were within 100 ± 10%, except for MAM-2201 with UHPSFC-MS/MS, and for UR-144 N-pentanoic acid and MAM-2201 N-4-OH-pentyl with UHPLC-MS/MS. Elution order obtained by UHPSFC-MS/MS was almost opposite to that obtained by UHPLC-MS/MS, making this instrument setup an interesting combination for screening and confirmation analyses in forensic cases. The UHPLC-MS/MS method has, since August 2014, been successfully used for confirmation of synthetic cannabinoids in urine samples revealing a positive immunoassay screening result.

http://dx.doi.org/10.1002/dta.1844

201. Evaluation Of Ultrahigh-Performance Supercritical Fluid Chromatography–Mass Spectrometry As An Alternative Approach For The Analysis Of Fatty Acid Methyl Esters In Aviation Turbine Fuel

Abstract
The current international reference method (IP585/10) for the determination of rapeseed methyl ester (RME) in jet fuel [aviation turbine fuel (AVTUR), current specifications U.S. ASTM 1655 and DEF STAN 91-91] uses gas chromatography–mass spectrometry (GC–MS). The fuel matrix requirements demand that a slow temperature gradient method (50 min) must be used. The fuel matrix also limits the application of this approach in relation to the detection and quantification of low-carbon-number fatty acid methyl esters (FAMEs), e.g., coconut methyl ester (CME), C8–C14 from coconut oil, a feedstock for FAME production in the Pacific Rim region. A 3 min ultrahigh-performance supercritical fluid chromatography–mass spectrometry (UHPSFC–MS) method has been developed for the analysis of RME and CME. This is compared to the existing reference method and an adapted form of the reference GC–MS method for the detection of low-carbon-number FAMEs. The UHPSFC–MS method is approximately 20 times faster than the ASTM reference method, affords a comparable linear dynamic range for the detection of total FAME
content up to 100 ppm with a linear correlation \( R^2 > 0.99 \) for RME, and is more suitable for the detection and quantification of lower chain length methyl esters.
http://dx.doi.org/10.1021/acs.energyfuels.5b00103

202. Analytical Advances In Pharmaceutical Impurity Profiling

Keywords: H. Lundbeck A/S, GlaxoSmithKline, pharmaceutical

Abstract

Impurities will be present in all drug substances and drug products, i.e. nothing is 100% pure if one looks in enough depth. The current regulatory guidance on impurities accepts this, and for drug products with a dose of less than 2 g/day identification of impurities is set at 0.1% levels and above (ICH Q3B(R2), 2006). For some impurities, this is a simple undertaking as generally available analytical techniques can address the prevailing analytical challenges; whereas, for others this may be much more challenging requiring more sophisticated analytical approaches. The present review provides an insight into current development of analytical techniques to investigate and quantify impurities in drug substances and drug products providing discussion of progress particular within the field of chromatography to ensure separation of and quantification of those related impurities. Further, a section is devoted to the identification of classical impurities, but in addition, inorganic (metal residues) and solid state impurities are also discussed. Risk control strategies for pharmaceutical impurities aligned with several of the ICH guidelines, are also discussed.
doi:10.1016/j.ejps.2015.12.007

203. Stereoselective Determination Of Tebuconazole In Water And Zebrafish By Supercritical Fluid Chromatography Tandem Mass Spectrometry

Keywords: Shenyang Agricultural University, tebuconazole enantiomers, zebrafish, food

Abstract

A simple and sensitive method for the enantioselective determination of tebuconazole enantiomers in water and zebrafish has been established using supercritical fluid chromatography (SFC)-MS/MS. The effects of the chiral stationary phases, mobile phase, auto back pressure regulator (ABPR) pressure, column temperature, flow rate of the mobile phase, and compensation pump solvent were evaluated. Finally, the optimal SFC-MS/MS working conditions were determined to include a CO₂/MeOH mobile phase (87:13, v/v), 2.0 mL/min flow rate, 2200 psi ABPR, and 30 °C column temperature using a Chiralpak IA-3 chiral column under electrospray ionization positive mode. The modified QuEChERS method was applied to water and zebrafish samples. The mean recoveries for the tebuconazole enantiomers were 79.8–108.4% with RSDs ≤ 7.0% in both matrices. The LOQs ranged from 0.24 to 1.20 μg/kg. The developed analytical method was further validated by application to the analysis of authentic samples.
http://dx.doi.org/10.1021/acs.jafc.5b02450
204. A Rapid Method For The Separation Of Vitamin D And Its Metabolites By Ultra-High Performance Supercritical Fluid Chromatography–Mass Spectrometry

Keywords: Lund University, vitamin_D2, vitamin_D3, plasma, health science

Abstract
In this study, a new supercritical fluid chromatography-mass spectrometry (SFC-MS) method has been developed for the separation of nine vitamin D metabolites within less than eight minutes. This is the first study of analysis of vitamin D and its metabolites carried out by SFC-MS. Six columns of orthogonal selectivity were examined, and the best separation was obtained by using a 1-aminoanthracene (1-AA) column. The number and the position of hydroxyl groups in the structure of the studied compounds as well as the number of unsaturated bonds determine the physiochemical properties and, thus the separation of vitamin D metabolites that is achieved on this column. All D2 and the D3 forms were baseline separated with resolution values>1.5. The effects of pressure, temperature, flow rate and different gradient modes were studied. Electro spray ionization (ESI) and atmospheric pressure chemical ionization (APCI) were compared in positive mode, both by direct infusion and after SFC separation. The results showed that the sensitivity in APCI(+) was higher than in ESI(+) using direct infusion. In contrast, the sensitivity in APCI(+) was 6-fold lower than in ESI(+) after SFC separation. The SFC-MS method was validated between 10 and 500ng/mL for all analytes with coefficient of determination (R(2))≥0.999 for all calibration curves. The limits of detection (LOD) were found to range between 0.39 and 5.98ng/mL for 24,25-dihydroxyvitamin D3 (24,25(OH)2D3) and 1-hydroxyvitamin D2 (1OHD2), respectively. To show its potential, the method was applied to human plasma samples from healthy individuals. Vitamin D3 (D3), 25-hydroxyvitamin D3 (25OHD3) and 24,25(OH)2D3 were determined in plasma samples and the concentrations were 6.6±3.0ng/mL, 23.8±9.2ng/mL and 5.4±2.7ng/mL, respectively.
doi:10.1016/j.chroma.2016.02.043

205. Application Of Ultra-High Performance Supercritical Fluid Chromatography For The Determination Of Carotenoids In Dietary Supplements

Keywords: Beijing Center for Disease Control and Prevention, food

Abstract
A quick and simple ultra-high performance supercritical fluid chromatography-photodiode array detector method was developed and validated for the simultaneous determination of 9 carotenoids in dietary supplements. The influences of stationary phase, co-solvent, pressure, temperature and flow rate on the separation of carotenoids were evaluated. The separation of the carotenoids was carried out using an Acquity UPC2 HSS C18 SB column (150 mm × 3.0 mm, 1.8 μm) by gradient elution with carbon dioxide and a 1:2 (v:v) methanol/ethanol mixture. The column temperature was set to 35 °C and the backpressure was 15.2 MPa. Under these conditions, 9 carotenoids and the internal standard, β-apo-8′-carotenal, were successfully separated within 10 min. The correlation coefficients ($R^2$) of the calibration curves were all above 0.997, the limits of detection for the 9 carotenoids were in the range of 0.33–1.08 μg/mL, and the limits of quantification were in the range of 1.09–3.58 μg/mL. The mean recoveries were from 93.4% to 109.5% at different spiking levels, and the relative standard deviations were between 0.8% and 6.0%. This method was successfully applied to the determination of 9 carotenoids in commercial dietary supplements. http://dx.doi.org/10.1016/j.chroma.2015.11.029

206. Evaluation Of Scale-Up From Analytical To Preparative Supercritical Fluid Chromatography

Keywords: Karlstadt University, AstraZeneca, SFC; Chiral separation; Transfer of method; Scale-up; Operational parameters; Design of experiments

Abstract

An approach for reliable transfer from analytical to preparative scale supercritical fluid chromatography was evaluated. Here, we accounted for the conditions inside the columns as well as to the fact that most analytical instruments are volume-controlled while most preparative scale units are mass-controlled. The latter is a particular problem when performing pilot scale experiments and optimizations prior to scaling up to production scale. This was solved by measuring the mass flow, the pressure and the temperature on the analytical unit using external sensors. Thereafter, it was revealed with a design of experiments approach that the methanol fraction and the pressure are the two most important parameters to control for preserved retention throughout the scale-up; for preserved selectivity the temperature was most important in this particular system. Using this approach, the resulting chromatograms from the preparative unit agreed well with those from the analytical unit while keeping the same column length and particles size. A brief investigation on how the solute elution volume varies with the volumetric flow rate revealed a complex dependency on pressure, density and apparent methanol content. Since the methanol content is a parameter of great importance to control during the scale up, we must be careful when changing operational and column design conditions which generates deviations in pressure, density and methanol content between different columns. http://dx.doi.org/10.1016/j.chroma.2015.11.001

207. Fast Separation Of Selected Cathinones And Phenylethylamines By Supercritical Fluid Chromatography

Keywords: Palacky University in Olomouc, University of Texas, Institute of Microbiology, v.v.i., designer drugs, BEH silica, BEH 2-ethylpyridine, CSH Fluoro-Phenyl, and HSS C18SB, Cathinones; Phenylethylamines; New designer drugs; Supercritical fluid chromatography; Electrospray ionization mass spectrometry

Abstract

The chromatographic behaviour of eleven synthetic cathinones and four phenylethylamines under supercritical/subcritical fluid conditions was investigated. Four stationary phases with sub-2 μm particles (Waters Acquity UPC2 BEH silica, BEH 2-ethylpyridine, CSH Fluoro-Phenyl, and HSS C18SB) were evaluated in terms of isomer resolution, chromatographic peak shape, and analysis time. Methanol, water, formic acid, ammonium hydroxide, ammonium acetate, and ammonium formate were mixed with carbon dioxide to test their influence on analyte retention and peak shapes. Methanol and ammonium cations were essential for successful separations. Efficient separations of four isomeric pairs ($R > 1$), and most of the remaining analytes, were achieved in less than 3.3 min on BEH and Fluoro-Phenyl columns with gradient of methanolic ammonium hydroxide in CO$_2$. Drugs were detected by positive electrospray ionization–triple quadrupole mass spectrometry in selected reaction monitoring mode. Added detection specificity and faster
separation of isomers on the BEH column using a steep gradient and high flow rate reduced analysis time of the mixture of 15 drugs to 1.6 min.
http://dx.doi.org/10.1016/j.chroma.2015.10.061

208. Method Development Approaches In Supercritical Fluid Chromatography Applied To The Analysis Of Cosmetics
2015 - Journal of Chromatography A

Keywords: U. Orleans, Groupe Yves Rocher, column screening, stationary phase, sunscreens, eye liner, eye serum, glyceryl caprylate, caffeine, UV filters, chemical materials

Abstract
Analyses of complex samples of cosmetics, such as creams or lotions, are generally achieved by HPLC. These analyses are often multistep gradients, due to the presence of compounds with a large range of polarity. For instance, the bioactive compounds may be polar, while the matrix contains lipid components that are rather non-polar, thus cosmetic formulations are usually oil-water emulsions. Supercritical fluid chromatography (SFC) uses mobile phases composed of carbon dioxide and organic co-solvents, allowing for good solubility of both the active compounds and the matrix excipients. Moreover, the classical and well-known properties of these mobile phases yield fast analyses and ensure rapid method development. However, due to the large number of stationary phases available for SFC and to the varied additional parameters acting both on retention and separation factors (co-solvent nature and percentage, temperature, backpressure, flow rate, column dimensions and particle size), a simplified approach can be followed to ensure a fast method development. First, suited stationary phases should be carefully selected for an initial screening, and then the other operating parameters can be limited to the co-solvent nature and percentage, maintaining the oven temperature and back-pressure constant. To describe simple method development guidelines in SFC, three sample applications are discussed in this paper: UV-filters (sunscreens) in sunscreen cream, glyceryl caprylate in eye liner and caffeine in eye serum. Firstly, five stationary phases (ACQUITY UPC(2)) are screened with isocratic elution conditions (10% methanol in carbon dioxide). Complementary of the stationary phases is assessed based on our spider diagram classification which compares a large number of stationary phases based on five molecular interactions. Secondly, the one or two best stationary phases are retained for further optimization of mobile phase composition, with isocratic elution conditions or, when necessary, two-step gradient elution. The developed methods were then applied to real cosmetic samples to assess the method specificity, with regards to matrix interferences, and calibration curves were plotted to evaluate quantification. Besides, depending on the matrix and on the studied compounds, the importance of the detector type, UV or ELSD (evaporative light-scattering detection), and of the particle size of the stationary phase is discussed.
http://dx.doi.org/10.1016/j.chroma.2015.10.053

209. Application Of Cinchona Alkaloid-Based Zwitterionic Chiral Stationary Phases In Supercritical Fluid Chromatography For The Enantioseparation Of N(Alpha)-Protected Proteinogenic Amino Acids
2015 - Journal of Chromatography A

Keywords: University of Szeged, University of Vienna, enantiomer, Zwitterionic chiral stationary phases; Nα-Fmoc-protected proteinogenic amino acids; Temperature effect, water

Abstract
Stereoselective supercritical fluid chromatographic separations of the enantiomers of a large set of Nα-Fmoc proteinogenic amino acids were carried out on the recently developed Cinchona alkaloid-based zwitterionic chiral stationary phases Chiralpak ZWIX(+)™ and ZWIX(-)™ with protic solvents as co-solvents. The effects of the mobile phase composition, the natures and concentrations of the acid or base additives, the co- and counter-ions and temperature on the separations were investigated. The retention time in most cases slightly increased, while the separation factor decreased with increasing temperature. The changes in standard enthalpy, Δ(ΔH°), entropy, Δ(ΔS°), and free energy, Δ(ΔG°), were calculated from the linear van't Hoff plots derived from the Inα vs 1/T curves in the studied temperature range (20-60°C). The values of the thermodynamic parameters depended on the natures of the selectors and the structures of the analytes. On both ZWIX(+)(™ and ZWIX(-)(™ columns, enthalpically-driven separations were observed. The elution sequence was determined in all cases and was observed to be opposite on ZWIX(+)™ and ZWIX(-)™ which acted for the presented applications as chiral anion exchanger.
210. An Attempt To Estimate Ionic Interactions With Phenyl And Pentafluorophenyl Stationary Phases In Supercritical Fluid Chromatography

Keywords: University of Orleans, method development, solvation parameter model, column classifications, separations science

Abstract
In several metabolomic studies, it has already been demonstrated that capillary electrophoresis hyphenated to mass spectrometry (CE-MS) can detect an important group of highly polar and ionized metabolites that are overseen by techniques such as NMR, LC-MS and GC-MS, providing complementary information. In this work, we present a strategy for anionic metabolite profiling by CE-MS using a cationic capillary coating. The polymer, abbreviated as PTH, is composed of a poly-(N,N,N',N'-tetraethyldiethylenetriamine, N-(2-hydroxypropyl) methacrylamide, TEDETAMA-co-HPMA (50:50) copolymer. A CE-MS method based on PTH-coating was optimized for the analysis of a group of 16 standard anionic metabolites. Separation was achieved within 12min, with high separation efficiency (up to 92,000 theoretical plates per meter), and good repeatability, namely, relative standard deviation values for migration times and peak areas were below 0.2 and 2.1%, respectively. The optimized method allowed the detection of 87 metabolites in orange juice and 142 metabolites in red wine, demonstrating the good possibilities of this strategy for metabolomic applications.

http://dx.doi.org/10.1016/j.chroma.2015.08.009

211. Performance Of The Same Column In Supercritical Fluid Chromatography And In Liquid Chromatography

Keywords: University of Pecs, mass transfer, stochastic model, moment analysis, separation science

Abstract
We have studied the chromatographic behavior of the homologous series of alkylbenzenes (ranging from octylbenzene to octadecylbenzene) on the same C18 reversed-phase column in supercritical fluid chromatography (SFC) and reversed phase liquid chromatography (RPLC) at various experimental conditions, such as different eluent compositions, flow-rates, and mobile phase densities. The first and the second moments of the peaks were used to estimate the overall mass-transfer processes in both chromatographic modes using the stochastic model of chromatography. The results confirm that in SFC - as the density of the mobile phase is influenced by the flow-rate - there is a broader variation of mass-transfer properties than in liquid chromatography. As expected, the optimum mobile phase velocity is higher in SFC, but there is no real difference in the minimum value of plate height, i.e. in the optimum efficiency

http://dx.doi.org/10.1016/j.chroma.2015.07.056

212. Sum-Of-Ranking-Differences To Rank Stationary Phases Used In Packed Column Supercritical Fluid Chromatography

Keywords: University of Orleans, University of Prague, Hungarian Academy of Sciences, column classification, method development, stationary phases, selectivity, separation science

Abstract
The identification of a suitable stationary phase in supercritical fluid chromatography (SFC) is a major source of difficulty for those with little experience in this technique. Several protocols have been suggested for column classification in high-performance liquid chromatography (HPLC), gas chromatography (GC), and SFC. However, none of the proposed classification schemes received general acceptance. A fair way to compare columns was proposed with the sum of ranking differences (SRD). In this project, we used the retention data obtained for 86 test compounds with varied polarity and structure, analyzed on 71 different stationary phases encompassing the full range in polarity of commercial packed columns currently available to the SFC chromatographer,
with a single set of mobile phase and operating conditions (carbon dioxide-methanol mobile phase, 25°C, 150bar outlet pressure, 3ml/min). First, a reference column was selected and the 70 remaining columns were ranked based on this reference column and the retention data obtained on the 86 analytes. As these analytes previously served for the calculation of linear solvation energy relationships (LSER) on the 71 columns, SRD ranks were compared to LSER methodology. Finally, an external comparison based on the analysis of 10 other analytes (UV filters) related the observed selectivity to SRD ranking. Comparison of elution orders of the UV filters to the SRD rankings is highly supportive of the adequacy of SRD methodology to select similar and dissimilar columns.

http://dx.doi.org/10.1016/j.chroma.2015.07.071

213. Response Surface Methodology For The Enantioseparation Of Dinotefuran And Its Chiral Metabolite In Bee Products And Environmental Sample By Supercritical Fluid Chromatography/Tandem Mass Spectrometry

Keywords: Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Institute for the Control of Agrochemicals, Ministry of Agriculture, chiral separation, enantiomers, environmental

Abstract

Tracing the enantiomers of dinotefuran and its metabolite in bee products and relevant environmental matrices is vital because of the high toxicity of their racemates to bees. In this study, a statistical optimization strategy using three-dimensional response surface methodology for the enantioseparation of dinotefuran and its metabolite UF was developed by a novel supercritical fluid chromatography/tandem mass spectrometry (SFC-MS/MS) technique. After direct evaluation of the chromatographic variables - co-solvent content, mobile phase flow rate, automated backpressure regulator pressure (ABPR), and column temperature - involved in the separation mechanism and assessment of the interactions among these variables, the optimal SFC-MS/MS working conditions were selected as a CO2/2% formic acid-methanol mobile phase, 1.9mL/min flow rate, 2009.8psi ABPR, and 26.0°C column temperature using an amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phase under electrospray ionization positive mode. Baseline resolution, favorable retention, and high sensitivity of the two pairs of enantiomers were achieved in pollen, honey, water, and soil matrices within 4.5min. Additionally, the parameters affecting the dispersive solid-phase extraction procedure, such as the type and content of extractant or purification sorbents, were systematically screened to obtain better extraction yields of the enantiomers. Mean recoveries were between 78.3% and 100.2% with relative standard deviations lower than 8.0% in all matrices. The limits of quantification ranged from 1.0μg/kg to 12.5μg/kg for the dinotefuran and UF enantiomers. Furthermore, the developed method was effectively applied to authentic samples from a market, an irrigation canal, and a trial field, and the enantioselective dissipation of dinotefuran and UF in soil was demonstrated.

http://dx.doi.org/10.1016/j.chroma.2015.07.067

214. Rapid Chiral Separation Of Atenolol, Metoprolol, Propranolol, And The Zwitterionic Metoprolol Acid Using Supercritical Fluid Chromatography-Tandem Mass Spectrometry – Application To Wetland Microcosms

Keywords: Uppsala University, National Veterinary Institute, Medical Products Agency, University of California – Berkeley, enantiomeric separation, chiralpak, separation science

Abstract

A method for enantiomeric separation of the three β-blocking agents atenolol, metoprolol, propranolol and the zwitterionic metoprolol acid, a major metabolite of both metoprolol and in environmental matrices also atenolol, has been developed. By use of supercritical fluid chromatography and the polysaccharide-based Chiralpak(®) IB-3, all four compounds were simultaneously enantiomerically separated (Rs>1.5) within 8min. Detection was performed using tandem mass spectrometry, and to avoid isobaric interference between the co-eluting metoprolol and metoprolol acid, the achiral column Acquity(®) UPC(2) BEH 2-EP was attached ahead of to the chiral column. Carbon dioxide with 18% methanol containing 0.5% (v/v) of the additives trifluoroacetic acid and ammonia in a 2:1 molar ratio were used as mobile phase. A post column make-up flow (0.3ML/min) of methanol containing 0.1% (v/v) formic acid was used to enhance the positive electrospray ionization. Detection was carried out using a triple quadrupole mass
spectrometer operating in the selected reaction monitoring mode, using one transition per analyte and internal standard. The method was successfully applied for monitoring the enantiomeric fraction change over time in a laboratory scale wetland degradation study. It showed good precision, recovery, sensitivity and low effect of the sample matrix.  
http://dx.doi.org/10.1016/j.chroma.2015.07.075


Keywords: University of Orleans, Servier Research Institute, column selection, method development, column screening, Nucleoshell, drugs, pharmaceuticals, acquity hss, column rankings

Abstract
Impurity profiling of organic products that are synthesized as possible drug candidates requires complementary analytical methods to ensure that all impurities are identified. Supercritical fluid chromatography (SFC) is a very useful tool to achieve this objective, as an adequate selection of stationary phases can provide orthogonal separations so as to maximize the chances to see all impurities. In this series of papers, we have developed a method for achiral SFC-MS profiling of drug candidates, based on a selection of 160 analytes issued from Servier Research Laboratories. In the first part of this study, focusing on mobile phase selection, a gradient elution with carbon dioxide and methanol comprising 2% water and 20mM ammonium acetate proved to be the best in terms of chromatographic performance, while also providing good MS response [1]. The objective of this second part was the selection of an orthogonal set of ultra-high performance stationary phases, that was carried out in two steps. Firstly, a reduced set of analytes (20) was used to screen 23 columns. The columns selected were all 1.7-2.5μm fully porous or 2.6-2.7μm superficially porous particles, with a variety of stationary phase chemistries. Derringer desirability functions were used to rank the columns according to retention window, column efficiency evaluated with peak width of selected analytes, and the proportion of analytes successfully eluted with good peak shapes. The columns providing the worst performances were thus eliminated and a shorter selection of columns (11) was obtained. Secondly, based on 160 tested analytes, the 11 columns were ranked again. The retention data obtained on these columns were then compared to define a reduced set of the best columns providing the greatest orthogonality, to maximize the chances to see all impurities within a limited number of runs. Two high-performance columns were thus selected: ACQUITY UPC² HSS C18 SB and Nucleoshell HILIC.  
http://dx.doi.org/10.1016/j.chroma.2015.07.035


Keywords: University of Orleans, Servier Research Institute, column selection, method development, column screening, Nucleoshell, drugs, pharmaceuticals, acquity hss, column rankings

Abstract
Supercritical fluid chromatography (SFC) is a very useful tool in the purpose of impurity profiling of drug candidates, as an adequate selection of stationary phases can provide orthogonal separations so as to maximize the chances to see all impurities. The purpose of the present work is to develop a method for chemical purity assessment. The first part, presented here, focuses on mobile phase selection to ensure adequate elution and detection of drug-like molecules, while the second part focuses on stationary phase selection for optimal separation and orthogonality. The use of additives in the carbon dioxide – solvent mobile phase in SFC is now commonplace, and enables in particular to increase the number of eluted compounds and to improve peak shapes. The objective of this first part was to test different additives (acids, bases, salts and water) for their chromatographic performance assessed in gradient elution with a diode-array detector, but also for the mass responses obtained with a single-quadrupole mass detector, equipped with an electrospray ionization source (Waters ACQUITY QDa).
In this project, we used a selection of one hundred and sixty compounds issued from Servier Research Laboratories to screen a set of columns and additives in SFC with a Waters ACQUITY UPC2 system. The selected columns were all high-performance columns (1.7–1.8 μm with totally porous particles or 2.6–2.7 μm with superficially porous particles) with a variety of stationary phase chemistries.

Initially, eight additives dissolved in the methanol co-solvent were tested on a UPC2 ACQUITY UPC2 HSS C18 SB column. A Derringer desirability function was used to classify the additives according to selected criteria: elution capability, peak shapes, UV baseline drift, and UV and mass responses (signal-to-noise ratios). Following these tests, the two best additives (ammonium acetate and ammonium hydroxide) were tested on a larger number of columns (10) where the two additives appeared to provide very comparable overall scores. However, ammonium acetate was selected for slightly better chromatographic quality.

In a second step, we investigated the effects of ammonium acetate concentration (between 0 and 25 mM in the methanol co-solvent) on retention and peak efficiency. Two types of silica supports were tested by working with ACQUITY UPC2 HSS C18 SB and BEH columns. 20 mM ammonium acetate in methanol with 2% water was finally selected as the best co-solvent composition.

The potential and limitations of on-line comprehensive reversed phase liquid chromatography × supercritical fluid chromatography for the separation of neutral compounds: an approach to separate an aqueous extract of bio-oil

Abstract

On-line comprehensive reversed phase liquid chromatography × supercritical fluid chromatography (RPLC × SFC) was investigated for the separation of complex samples of neutral compounds. The presented approach aimed at overcoming the constraints involved by such a coupling. The search for suitable conditions (stationary phases, injection solvent, injection volume, design of interface) are discussed with a view of ensuring a good transfer of the compounds between both dimensions, thereby allowing high effective peak capacity in the second dimension. Instrumental aspects that are of prime importance in on-line 2D separations, were also tackled (dwell volume, extra column volume and detection). After extensive preliminary studies, an on-line RPLC × SFC separation of a bio-oil aqueous extract was carried out and compared to an on-line RPLC × RPLC separation of the same sample in terms of orthogonality, peak capacity and sensitivity. Both separations were achieved in 100 min. For this sample and in these optimized conditions, it is shown that RPLC × SFC (with Hypercarb and Acquity BEH-2EP as stationary phases in first and second dimension respectively) can generate a slightly higher peak capacity than RPLC × RPLC (with Hypercarb and Acquity CSH phenyl-hexyl as stationary phases in first and second dimension respectively) (620 vs 560). Such a result is essentially due to the high degree of orthogonality between RPLC and SFC which may balance for lesser peak efficiency obtained with SFC as second dimension. Finally, even though current limitations in SFC instrumentation (i.e. large extra-column volume, large dwell volume, no ultra-high pressure) can be critical at the moment for on-line 2D separations, RPLC × SFC appears to be a promising alternative to RPLC × RPLC for the separation of complex samples of neutral compounds.

Use of isopycnic plots to understand the role of density in SFC, which is now a misnomer. In this report we intentionally refrained from using the expansion anywhere to avoid any technical inaccuracy. - I. effect of pressure variation on retention factors

Abstract

This paper aims to demonstrate the effect of pressure variations in modifying analyte retention behavior in SFC. There is a general understanding that in SFC increasing pressure decreases the retention factor (k'), and vice versa. What is not clearly discussed or explained in any recent literature is that these variations can be very different at different operating pressures,
temperatures and modifier concentrations. It is important to have a clearer understanding on these variabilities during method development and results analysis. In this paper the nature of k’ variation with pressure, at different temperatures and modifier concentrations, will be explained with the help of isopycnic plots of CO2 and CO2+methanol mixtures.

http://dx.doi:10.1016/j.chroma.2015.05.052

219. A Closer Study Of Peak Distortions In Supercritical Fluid Chromatography As Generated By The Injection 2015 - Journal of Chromatography A

Keywords: Karlstadt University, University of Western Sydney, viscous fingering, solvent strength, modeling, tracer peak, adsorption isotherms, separation science

Abstract
In SFC the sample cannot be dissolved in the mobile phase, so it is often dissolved in pure modifier, or another liquid, sometimes resulting in serious distortions of the eluted peak profiles already at moderately high injection volumes. It is suspected the reasons for these effects are solvent strength mismatch and/or viscosity mismatch. This study presents a systematic and fundamental investigation of the origin of these peak deformations due to the injection solvent effects in SFC, using both systematic experiments and numerical modeling. The first set of experiments proved that the injection volume and the elution strength of the sample solution had a major impact of the shapes of the eluted peaks. Secondly, the sample band elution profile was numerically modeled on a theoretical basis assuming both un-retained and retained co-solvent injection plugs, respectively. These calculations quantitatively confirmed our first set of experiments but also pointed out that there is also an additional significant effect. Third, viscous fingering experiments were performed using viscosity contrast conditions imitating those encountered in SFC. These experiments clearly proved that viscous fingering effects play a significant role. A new method for determination of adsorption isotherms of solvents was also developed, called the “Retention Time Peak Method” (RTPM). The RTPM was used for fast estimation of the adsorption isotherms of the modifier and required using only two experiments.

http://dx.doi.org/10.1016/j.chroma.2015.04.059

220. Chromatographic Resolution Of Atropisomers For Toxicity And Biotransformation Studies In Pharmaceutical Research 2015 - Journal of Chromatography A

Keywords: Pfizer, chiral chromatography, stability testing, energy barrier calculation, absolute configuration, human plasma, pharmaceutical

Abstract
Atropisomerism can be a complex concept for those who have not encountered it before. This paper discusses the experiments for identification, isolation, thermal stability, toxicity and biotransformation of various species. The identified atropisomers are a series of rotational hindered biaryl, rotational hindered amide, ring flip, and macrocycles atropisomers identified using supercritical fluid chromatography (SFC) and high performance liquid chromatography (HPLC). These technologies offered the advantage of separating various atropoanantiomers, atropdiastereomers and mixed atropisomers with other forms of stereoisomers in both analytical and preparative scales. With ultra-performance convergence chromatography (UPC(2)), the detection of N-oxide atropisomer metabolites can be obtained at very low level thus enabling the observation of conversion in human plasma possible. As the resolution of atropisomers are related to the energy barriers on the rotational axis, a calculated computational protocol was developed to predict the formation. A threshold of 10kcal/mol was established for possible detection of the atropisomers’ existence with chromatographic technologies at room temperature or above. The atropisomer with higher energy barrier (>20kcal/mol) were isolated via preparative chromatography and the isolates studied in vitro and in vivo for evaluation of their stability in human plasma. The detailed analytical method development to analyze the biotransformation of the atropisomers in human plasma are also discussed in this paper.

http://dx.doi.org/10.1016/j.chroma.2015.04.023

221. Exploring The Enantioseparation Of Amino-Nphthol Analogues By Supercritical Fluid Chromatography
Abstract
The direct separation of the enantiomers of 1-(α-aminoarylmethyl)-2-naphthol, 1-(α-aminoalkyl)-2-naphthol, 2-(α-aminoarylmethyl)-1-naphthol analogues and 2-(1-amino-2-methyl(propyl)-1-naphthol) was investigated in supercritical fluid chromatography. Five commercially available chiral stationary phases based on immobilized polysaccharide chiral selectors (Chiralpak IA, IB, IC, ID and IE) were evaluated. Chiralpak IB was by far the most efficient to achieve the separation of these racemates and was further selected for optimization of elution conditions. The effects of column temperature (varying between 5 and 45 °C) and co-solvent added to carbon dioxide (methanol, ethanol, isopropanol and dichloromethane) were investigated. A particular attention was paid to mobile-phase additives. Several of them, acids, bases or salts (namely water, formic acid, acetic acid, trifluoroacetic acid, diethylamine, diethanolamine, triethylamine, triethanolamine, dimethylethanolamine, ammonia and ammonium acetate), were tested in order to improve peak shapes while maintaining selectivity. With the help of other achiral naphthol derivatives, the additive effects were examined.

222. Search For Improved Fluorinated Stationary Phases For Separation Of Fluorine-Containing Pharmaceuticals From Their Desfluoro Analogs
Abstract
Evaluation of several fluorine-containing stationary phases for the chromatographic separation of fluorine-containing pharmaceuticals from their corresponding desfluoro analogs revealed a number of perfluoroaryl and perfluoroalkyl stationary phases that afford good separations. These fluororous stationary phases exhibit greater retention for the fluorine-containing compounds relative to the H-containing analogs, consistent with a fluorophilic retention mechanism. While both perfluoroalkyl and perfluoroaryl stationary phases afford adequate resolution, the perfluoroaryl columns generally exhibit superior separation factor (α) and peak efficiency (N), resulting in faster baseline separations, with the Hypersil Gold PFP and Poroshell 120 PFP columns providing the best overall performance for the test group studied.

223. The Many Faces Of Packed Column Supercritical Fluid Chromatography – A Critical Review
Abstract
Packed column sub- and supercritical fluid chromatography (SFC) is a versatile separation method: on the one hand the number of parameters acting on the quality of a separation is very large, and the effects of these parameters can be complex (and not always fully understood). But on another hand, due to numerous advantageous properties of CO2-based mobile phases, method development is a fast task. This paper is a review of the main features of SFC, focusing essentially on achiral separations. However, several fundamental aspects discussed here are also relevant to chiral SFC separations. This is not intended to be an extensive review, as the way to practice SFC has somewhat evolved with time. We rather wished to provide an expert opinion on the characteristics of the method, pointing at the sources of difficulty and displaying the wide possibilities that it offers. A large number of selected applications concerning several different areas are also presented.
224. Expanding The Potential Of Chiral Chromatography For High-Throughput Screening Of Large Compound Libraries By Means Of Sub–2 Mm Whelk-O 1 Stationary Phase In Supercritical Fluid Conditions

Keywords: University of Rome, Novartis, University of Ferrara, University of Naples, Regis Technologies, chiral separations, enantioselective screening, separation science

Abstract
With the aim of exploring the potential of ultra-fast chiral chromatography for high-throughput analysis, the new sub-2 micron Whelk-O 1 chiral stationary phase (CSP) has been employed in supercritical fluid conditions to screen 129 racemates, mainly of pharmaceutical interest. By using a 5-cm long column (0.46cm internal diameter), a single co-solvent (MeOH) and a 7-min gradient elution, 85% of acidic and neutral analytes considered in this work have been successfully resolved, with resolution (Rs) larger than 2 in more than 65% of cases. Moreover, almost a half of basic samples that, for their own characteristics, are known to be difficult to separate on Whelk-O 1 CSP, have shown Rs greater than 0.3. The screening of the entire library could be accomplished in less than 24h (single run) with 63% of positive score. For well-resolved enantiomers (Rs roughly included between 1 and 3), we show that method transfer from gradient to isocratic conditions is straightforward. In many cases, isocratic ultra-fast separations (with analysis time smaller than 60s) have been achieved by simply employing, as isocratic mobile phase, the eluent composition at which the second enantiomer was eluted in gradient mode. By considering the extension and variety of the library in terms of chemico-physical and structural properties of compounds and numerosness, we believe that this work demonstrates the real potential of the technique for high-throughput enantioselective screening.

http://dx.doi.org/10.1016/j.chroma.2015.01.042

225. Possibilities Of Retention Modeling And Computer Assisted Method Development In SFC

Keywords: Vrije Universiteit Brussels, University of Geneva, method development, retention modeling, retention prediction, separation science

Abstract
The multi-modal retention mechanism in supercritical fluid chromatography (SFC) results in a non-linear dependency of log(k) on the fraction of organic solvent φ and log(φ). In the present study, the possibility of retention modeling for method development purposes in SFC was investigated, considering several non-linear isocratic relationships. Therefore, both isocratic and gradient runs were performed, involving different column chemistries and analytes possessing diverse physico-chemical properties. The isocratic retention data of these compounds could be described accurately using the non-linear retention models typically used in HILIC and reversed-phase LC. The interconversion between isocratic and gradient retention data was found to be less straightforward than in RPLC and HILIC because of pressure effects. The possibility of gradient predictions using gradient scouting runs to estimate the retention parameters was investigated as well, showing that predictions for other gradients with the same starting conditions were acceptable (always below 5%), whereas prediction errors for gradients with a different starting condition were found to be highly dependent on the compound. The second part of the study consisted of the gradient optimization of two pharmaceutical mixtures (one involving atorvastatin and four related impurities, and one involving a 16 components mixture including eight drugs and their main phase I metabolites). This could be done via individual retention modeling based on gradient scouting runs. The best linear gradient was found via a grid search and the best multi-segment gradient via the previously published one-segment-per-component search. The latter improved the resolution between the critical pairs for both mixtures, while still giving accurate prediction errors (using the same starting concentrations as the gradient scouting runs used to build the model). The optimized separations were found in less than 3 h and 8 h of analysis time (including equilibration times), respectively.

http://dx.doi.org/10.1016/j.chroma.2014.12.077

226. Study Of Ultrahigh Performance Supercritical Fluid Chromatography To Measure Free Fatty Acids Without Fatty Acid Ester Preparation

2015 - Journal of Chromatography B
Abstract
Most lipids are best characterized by their fatty acids which may differ in (a) chain length, (b) degree of unsaturation, (c) configuration and position of the double bonds, and (d) the presence of other functionalities. Thus, a fast, simple, and quantitative analytical technique to determine naturally occurring free fatty acids (FFA) in different samples is very important. Just as for saponified acylglycerols, the determination of FFA's has generally been carried out by high resolution gas chromatography (HRGC). The use of an open tubular capillary column coupled with a flame ionization or mass spectrometric detector provides for both high resolution and quantification of FFA's but only after conversion of all free fatty acids to fatty acid methyl esters (FAME) or pentafluorobenzyl esters. Unfortunately, volatilization of labile ester derivatives of mono- and poly-unsaturated FFA's can cause both thermal degradation and isomerization of the fatty acid during HRGC. The employment of a second generation instrument (here referred to as UltraHigh Performance Supercritical Fluid Chromatograph, UHPSFC) with high precision for modified flow and repeated back pressure adjustment in conjunction with sub-2μm various bonded silica particles (coupled with evaporative light scattering, ELSD, and mass spectrometric, MS, detection) for separation and detection of the following mixtures is described: (a) 31 free fatty acids, (b) isomeric FFA's, and (c) lipophilic materials in two real world fish oil samples. Limits of detection for FFA's via UHPSFC/MS and UHPSFC/ELSD versus detection of FAME's via HRGC/MS are quantitatively compared.

http://dx.doi.org/10.1016/j.jchromb.2015.05.031

227. Simultaneous Determination Of Seven Gestagens In Kidney Fats By Ultraperformance Convergence Chromatography Tandem Mass Spectrometry

2015 - Journal of Chromatography B

Keywords: Huazhong Agricultural University, Wangeningen University, gestagens, kidney fat, health science

Abstract
An ultra-performance convergence chromatography (UPC2) system coupled tandem mass spectrometry was successfully utilised to analyse chlormadinone acetate, delmadinone acetate, fluorogestone acetate, medroxyprogesterone acetate, megestrol acetate, melengestrol acetate, chlorosteasterone acetate in bovine and porcine kidney fat. This novel approach obtained an improved resolution in comparison to previously reported chromatographic methods combined with MS detector in a shorter analytical time. All the acetylgestagen compounds were well separated on a ACQUITY UPC(2) HSS C18 column (3.0 × 100 mm, 1.7 μm) by applying methanol and carbon dioxide (2/98). The LOQ of delmadinone acetate, melengestrol acetate, medroxyprogesterone acetate and megestrol acetate are 0.5 μg/kg, fluorogestone acetate, chlormadinone acetate and chlorosteasterone acetate 1.0 μg/kg. The recoveries of gestagens spiked in kidney fats at a concentration range of 0.5 to 4 μg/kg were above 86.1% with relative standard deviations (RSD) less than 13.1%. These rapid and reliable methods can be used to efficiently separate, characterize and quantify the residues of gestagens in kidney fats with advantages of shorter time, more sensitive and environmental friendly.

http://dx.doi.org/10.1016/j.jchromb.2015.02.034

228. Ultrahigh Performance Supercritical Fluid Chromatography Of Lipophilic Compounds With Application To Synthetic And Commercial Biodiesel

2015 - Journal of Chromatography B

Keywords: Virginia Tech, Waters, tobacco seed oil, biodiesel, soybean oil, bound and free glycerols, chemical materials

Abstract
Ultrahigh performance supercritical fluid chromatography (UHPSFC) in combination with sub-2μm particles and either diode array ultraviolet (UV), evaporative light scattering, (ELSD), or mass spectrometric (MS) detection has been shown to be a valuable technique for the determination of acylglycerols in soybean, corn, sesame, and tobacco seed oils. Excellent resolution on an un-endcapped single C18 column (3.0mm×150mm) with a mobile phase gradient of acetonitrile and carbon dioxide in as little as 10min served greatly as an improvement on first generation packed column SFC instrumentation. Unlike high resolution gas chromatography and high performance
liquid chromatography with mass spectrometric detection, UHPSFC/MS was determined to be a superior analytical tool for both separation and detection of mono-, di-, and tri-acylglycerols as well as free glycerol itself in biodiesel without derivatization. Baseline separation of residual tri-, di-, and mono-acylglycerols alongside glycerol at 0.05% (w/w) was easily obtained employing packed column SFC. The new analytical methodology was applied to both commercial B100 biodiesel (i.e. fatty acid methyl esters) derived from vegetable oil and to an "in-house" synthetic biodiesel (i.e. fatty acid ethyl esters) derived from tobacco seed oil and ethanol both before and after purification via column chromatography on bare silica. http://dx.doi.org/10.1016/j.jchromb.2014.12.012

229. Supercritical Fluid Chromatography With Diode-Array Detection For Emerging Contaminants Determination In Water Samples. Method Validation And Estimation Of The Uncertainty

Keywords: Universidad Colima, Viridis, Bond Elut, wastewater, pesticide, bactericide, pharmaceuticals

Abstract
Here we present a communication about the article “Salvatierra-Stamp VC, Ceballos-Magaña SG, Gonzalez J, Ibarra-Galván V, Muñiz-Valencia R (2015) Analytical method development for the determination of emerging contaminants in water using supercritical-fluid chromatography coupled with diode-array detection. Analytical and Bioanalytical Chemistry 407:4219-4226”. In this paper, a selective, linear, accurate and precise supercritical-fluid chromatography coupled with diode-array detection method was developed and validated for the determination of seven emerging contaminants: two pharmaceuticals, three endocrine disruptors, one bactericide and one pesticide. The compounds were base-line separated in around 10 minutes. Also, the method involved a sample treatment optimization by means of C18-OH solid phase extraction cartridges. The developed method was validated. In this sense, the correlation coefficient and recovery was higher than 0.9997 and 94%, respectively. Limit of detection and quantification was in the range of 0.10-1.59 μg/L and 0.31-4.83 μg/L, respectively. The measurement uncertainty was evaluated using the top-down model considering six sources of uncertainty. For all compounds, the uncertainty associated with accuracy and linearity regression was the main contribution to the combined uncertainty. Expanded uncertainties for each compound in method analysis were lower than 10.8%. Finally the method was successfully applied to environmental water samples. http://dx.doi.org/10.4172/2157-7064.100029

230. Development Of A Sensitive And Rapid Method For Rifampicin Impurity Analysis Using Supercritical Fluid Chromatography

Keywords: China Pharmaceutical University, Ministry of Education, Anhui Institute for Food and Drug Control, impurities, pharmaceutical

Abstract
A novel simple, fast and efficient supercritical fluid chromatography (SFC) method was developed and compared with RPLC method for the separation and determination of impurities in rifampicin. The separation was performed using a packed diol column and a mobile phase B (modifier) consisting of methanol with 0.1% ammonium formate (w/v) and 2% water (v/v). Overall satisfactory resolutions and peak shapes for rifampicin quinone (RQ), rifampicin (RF), rifamycin SV (RSV), rifampicin N-oxide (RNO) and 3-formylrifamycinSV (3-FR) were obtained by optimization of the chromatography system. With gradient elution of mobile phase, all of the impurities and the active were separated within 4 min. Taking full advantage of features of SFC (such as particular selectivity, non-sloping baseline in gradient elution, and without injection solvent effects), the method was successfully used for determination of impurities in rifampicin, with more impurity peaks detected, better resolution achieved and much less analysis time needed compared with conventional reversed-phase liquid chromatography (RPLC) methods. http://dx.doi:10.1016/j.jpba.2015.06.012
231. Method Development For Impurity Profiling In SFC: The Selection Of A Dissimilar Set Of Stationary Phases

Abstract
Supercritical fluid chromatography (SFC) is drawing considerable interest as separation technique in the pharmaceutical industry. The technique is already well established in chiral separations both analytically and on a preparative scale. The use of SFC as a technique for drug impurity profiling is examined here. To define starting conditions in method development for drug impurity profiling, a set of dissimilar stationary phases is screened in parallel. The possibility to select a set of dissimilar columns using the retention factors (k-values) for a set of 64 drugs measured on 27 columns in SFC was examined. Experiments were carried out at a back-pressure of 150 bar and 25 °C with a mobile phase consisting of CO2 and methanol with 0.1% isopropylamine (5-40% over 10 min) at a flow rate of 3 mL/min. These k-values were then used to calculate correlation coefficients on the one hand and to perform a principal component analysis on the other. The Kennard and Stone algorithm, besides dendrograms and correlation-coefficient colour maps were used to select a set of 6 dissimilar stationary phases. The stationary phase characterization results from this study were compared to those from previous studies found in the literature. Retention mechanisms for compounds possessing different properties were also evaluated. The dissimilarity of the selected subset of 6 stationary phases was validated using mixtures of compounds with similar properties and structures, as one can expect in a drug impurity profile.

http://dx.doi.org/10.1016/j.jpba.2014.12.043

232. Supercritical Fluid Chromatography For The Separation Of Isoflavones

Abstract
The first protocol for the analysis of isoflavones by supercritical fluid chromatography is reported. Optimum results were obtained on an Acquity UPC(2) BEH 1.7 μm column, using a solvent gradient of supercritical carbon dioxide and methanol (with phosphoric acid as additive) for elution. The method enables the baseline separation of nine isoflavones (aglyca and glycosides) in 8 min, and is suitable for their quantitative determination in dietary supplements containing soy (Glycine max), red glove (Trifolium pratense) and kudzu (Pueraria lobata). Method validation confirmed that the assay is selective, linear (R(2)≥0.9994), accurate (recovery rates from 97.6 to 102.4%), as well as precise on the short- and long-term level (intra-day precision ≤2.1%), and shows an on-column detection limit of 0.2 ng and below. This, together with an excellent performance shown in the analysis of real samples, indicates that SFC is well suited for the fast and accurate determination of isoflavones in complex matrices. Disadvantages compared to the established approaches were not observed, so that SFC has to be considered in this case as an (at least) equivalent analytical alternative.

http://dx.doi.org/10.1016/j.jpba.2015.01.013

233. Supercritical Fluid Chromatography In Pharmaceutical Analysis

Abstract
In the last few years, there has been a resurgence of supercritical fluid chromatography (SFC), which has been stimulated by the introduction of a new generation of instruments and columns from the main providers of chromatographic instrumentation, that are strongly committed to advancing the technology. The known limitations of SFC, such as weak UV sensitivity, limited reliability and poor quantitative performance have been mostly tackled with these advanced instruments. In addition, due to the obvious benefits of SFC in terms of kinetic performance and its complementarity to LC, advanced packed-column SFC represents today an additional strategy in the toolbox of the analytical scientist, which may be particularly interesting in pharmaceutical analysis. In the present review, the instrumentation and experimental conditions (i.e. stationary
phase chemistry and dimensions, mobile phase nature, pressure and temperature) to perform "advanced SFC" are discussed. The applicability of SFC in pharmaceutical analysis, including the determination of drugs in formulations and biofluids is critically discussed. http://dx.doi.org/10.1016/j.jpba.2015.03.007

234. Advances In The Analytical Methodologies: Profiling Steroids In Familiar Pathways-Challenging Dogmas

2015 - The Journal of Steroid Biochemistry and Molecular Biology

Keywords: University of Stellenbosch, 11βHSD, PCOS, LNCaP prostate cancer, H295R adrenal cell, steroid metabolites, steroidogenesis, health science

Abstract

The comprehensive evaluation of the adrenal steroidogenic pathway, given its complexity, requires methodology beyond the standard techniques currently employed. Advances in LC–MS/MS, coupled with in vitro cell models that produce all the steroid metabolites of the mineralo-, glucocorticoid and androgen arms, present a powerful approach for the comprehensive evaluation of adrenal steroidogenesis in response to compounds of interest including bioactives, drug treatments and endocrine disrupting compounds. UHPLC–MS/MS analysis of steroid panels in forskolin, angiotensin II and K+ stimulated H295R cells provides a snapshot of their effect on intermediates and end products of adrenal steroidogenesis. The impact of full steroid panel evaluations by LC– and GC–MS/MS extends to clinical profiling with the characterization of normal pediatric steroid reference ranges in sexual development and of disease-specific profiles improving diagnosis and sub classification. Comprehensive analyses of steroid profiles may potentially improve patient outcomes together with the application of treatments specifically suited to clinical subgroups. LC–MS/MS and GC–MS/MS applications in the analyses of comprehensive steroid panels are demonstrated in clinical conditions such as congenital adrenal hyperplasia in newborns requiring accurate diagnoses and in predicting metabolic risk in polycystic ovary syndrome patients. Most notable perhaps is the impact of LC–MS/MS evaluations on our understanding of the basic biochemistry of steroidogenesis with the detection of the long forgotten adrenal steroid, 11β-hydroxyandrostenedione, at significant levels. The characterization of its metabolism to androgen receptor ligands in the LNCaP prostate cancel cell model, specifically within the context of recurring prostate cancer, lends new perspectives to old dogmas. We demonstrate that UHPLC–MS/MS has enabled the analyses of novel metabolites of the enzymes, SRD5A, 11βHSD and 17βHSD, in LNCaP cells. Undoubtedly, the continuous advances in the analytical methodologies used for steroid profiling and quantification will give impetus to the unraveling of the remaining enigmas, old and new, of both hormone biosynthesis and metabolism. http://dx.doi.org/10.1016/j.jsbmb.2015.04.009
235. Rapid And Simultaneous Analysis Of Sesquiterpene Pyridine Alkaloids From *Tripterygium Wilfordii* Hook. F. Using Supercritical Fluid Chromatography-Diode Array Detector-Tandem Mass Spectrometry

2015 - The journal of Supercritical Fluids

**Keywords:** East China University, Sesquiterpene pyridine alkaloids, *Tripterygium wilfordii* Hook. F, component profiling, natural products, tcm, health science

**Abstract**

Sesquiterpene pyridine alkaloids are considered to be the active components of *Tripterygium wilfordii* Hook. f. A rapid method was developed for comprehensive profiling of sesquiterpene pyridine alkaloids from an extract of root bark of *T. wilfordii* using supercritical fluid chromatography-diode array detector-tandem mass spectrometry (SFC-DAD-MS/MS). Alkaloids were separated on a BEH 2EP column within 10 min, eluted by CO₂-methanol as mobile phase with a back pressure of 13.8 Mpa and a column temperature of 45 °C. MS/MS analysis of [M + H]⁺ ion of each alkaloid standard showed that all the pyridine alkaloids produced very similar fragmentation patterns. The product-ions at m/z 206 and 204 were identified as the diagnostic fragments while mass region of 200–500 Da was assigned as the characteristic region. As a result, 71 components in the extract were identified as sesquiterpene pyridine alkaloids, including 40 wilfordate/evoninate type alkaloids, 13 iso-wilfordate/evoninate type alkaloids and 19 hydroxyl-wilfordate/evoninate type alkaloids. The results proved the feasibility of SFC-DAD-MS/MS method for the rapid and high-throughput analysis of sesquiterpene pyridine alkaloids in complex samples.

http://dx.doi.org/10.1016/j.supflu.2015.05.006

236. Applicability Of Ultra Performance Convergence Chromatography - A New Generation Of Supercritical Fluid Chromatography For The Analysis Of Pesticide Residues

2015 - Periodica Polytechnica Chemical Engineering

**Keywords:** Corvinus University of Budapest, Budapest University of Technology and Economics, food safety, quechers, food

**Abstract**

Monitoring and controlling wide variety of pesticide residues is a crucial challenge of food safety. In our study ultra-performance convergent chromatography (UPC²), as the new generation of supercritical fluid chromatography coupled with ESI-MS/MS system was applied to separate a set of pesticides to investigate their chromatographic behavior under various UPC² conditions. 30 components were selected representing the GC and LC measurable components. Capacity factors obtained from LC and GC runs UPC²-PDA were compared. Based on our data UPC² should be considered as an alternative chromatographic approach with separation mechanisms not yet fully characterized. Interestingly the type of mobile phase modifier influences the ionization in an ESI-MS system.

http://dx.doi.org/10.3311/PPch.8363

237. Exploring The Complexity Of Oil Sands Process-Affected Water By High Efficiency Supercritical Fluid Chromatography/Orbitrap Mass Spectrometry

2015 - Rapid Communications in Mass Spectrometry

**Keywords:** University of Alberta, dissolved organic compounds, water contamination, water treatment, napthenic acid, environmental

**Abstract**

Approximately 1 billion m³ of oil sands process-affected water (OSPW) is currently stored in tailings ponds in Northern Alberta, Canada. The dissolved organic compounds in OSPW have been termed a *supercomplex* mixture of bitumen-derived substances and continuing efforts to understand its underlying chemical composition are important for evaluating its environmental hazards. This combination of high efficiency chromatography and ultra-high mass resolution detection resulted in a powerful method with capabilities for characterizing or 'fingerprinting' unknown species with little interference. The method has great promise for environmental monitoring and forensics in the oil sands region, as well as for further studies on the composition of dissolved organic compounds in OSPW.
238. Quantitative Analysis Of Five Toxic Alkaloids In Aconitum Pendulum By Ultra-Performance Convergence Chromatography (UPC²) Coupled With Mass Spectrometry

Keywords: Lanzhou Institute of Chemical Physics, tcm, biobotanicals, herbal supplements, medicinal herbs, method development, method validation, food

Abstract
A rapid and efficient ultra-performance convergence chromatography (UPC²) method coupled with electrospray ionization single quadrupole mass spectrometry (ESI-MS) was developed and validated for the simultaneous quantification of five diester diterpenoid alkaloid constituents (3-acetylaconitine, hypaconitine, deoxyaconitine, mesaconitine, aconitine) in Aconitum pendulum. Optimum separation was achieved on a BEH 2-EP C18 column (2.1 × 150 mm i.d., 1.7 μm particle) with a gradient elution of a mixture of A (supercritical CO₂) and B (methanol containing 10 mmol L⁻¹ ammonium acetate) and at a flow rate of 0.8 mL min⁻¹ within 3 minutes. Quantification was performed using mass spectrometry in a positive ion ionization mode and selected ion recording (SIR) mode. The influences of column, modifier, additive, column temperature, and back pressure were investigated. The five alkaloids were identified and quantified using a comparison of retention time, ultraviolet spectrum, molecular ion peak (obtained from a selective ion recording mode) and peak areas with the reference compounds. The method was validated through linearity, limits of detection, limits of quantification, precision, stability, repeatability, and accuracy. The validated method was applied to analyze A. pendulum, which provided a reference for the quality evaluation of A. pendulum.

http://dx.doi.org/10.1039/C5RA21233B

239. Analysis Of Anthraquinones In Rhubarb (Rheum Palmatum And Rheum Officinale) By Supercritical Fluid Chromatography

Keywords: University of Innsbruck, chrysophanol, physcion, emodin, aloemodin, and rhein, Rhubarb; Anthraquinone, food

Abstract
The first report on the separation of five anthraquinones (chrysophanol, physcion, emodin, aloemodin, and rhein) from rhubarb by supercritical fluid chromatography indicates that this technique is an interesting analytical alternative not just for non-polar substances. Within less than five minutes the compounds could be baseline resolved, using a mobile phase comprising supercritical carbon dioxide and methanol with 0.05% diethylamine. The optimum stationary phase showed to be an Acquity UPC² HSS C18 SB 1.8 μm column, operated at a flow rate of 2 ml/min and a temperature of 30 °C. Method validation confirmed that the developed procedure is selective, linear (R²≥0.999), accurate (recovery rates: 95.4% to 103.1%), and precise (intra-day≤6.9%, inter-day≤4.7%); the limit of detection was below 0.5 ng on-column. The analysis of plant extracts was feasible with acceptable repeatability (σrel≤3.8%), and it determined 0.3 to 0.7% of free aglyca in the native samples. After hydrolysis according to the European Pharmacopoeia, a rise in the total content up to 2.1% was observed, with rhein being the most dominant derivative in nearly all specimens.

http://dx.doi.org/10.1016/j.talanta.2015.08.011
Abstract
The chiral alkynylcarbinol motif typically found in natural marine products, has been the subject of intense research activity for its pharmacophoric properties, in particular cytotoxicity against tumor cell lines. In a chemical synthesis-driven four-parameter structure–activity relationship (SAR) study from the (S,E)-eicos-4-en-1-yl-3-ol natural reference 1, the (S)-dialkynylcarbinol unit of the non-natural dehydro derivative 2 emerged as an unprecedented anti-tumoral pharmacophore. An extended study of lipidic alkynylcarbinol pharmacophores is presented, addressing additional structural parameters: Z→E isomerization of the alkenyl carbinol substituent of 1, variation of the lipidic chain length of 2 (C₃n, n=3, 4, 6), oxidation or substitution of the carbinol unit of 2 (to ketone, tertiary methylcarbinol, or methylether), cyclomethylenation of the double bond of 1. The synthesis of these analogues is described, including the preparation of enantio-enriched chiral alkynylcarbinol derivatives using a modified Carreira procedure for Zn-mediated addition of (trialkysilyl)acetylene substrates to ynals in the presence of (−)- or (+)-N-methylephedrine. Preliminary cytotoxicity evaluation of 12 new products against the HCT 116 tumor cell line are finally reported and the results compared with those obtained for 1 and 2. These observations support and refine the relevance of the pharmacophoric character of secondary DAC units.

http://dx.doi.org/10.1016/j.tet.2015.08.003
review concludes with a discussion on the major challenges that exist in clinical lipidomics studies and looks at potential solutions that can address them.
http://dx.doi.org/10.1016/j.trac.2014.10.010

242. Qualitative And Quantitative Analysis Of Bushen Jiannao Grains By Ultra Performance Convergence Chromatography

Keywords: Bushen Jianno Grains; Ultra performance convergence chromatography; echinacoside

Abstract
An ultra performance convergence chromatographic (UPC²) method was established for the attribution analysis of the main peaks as well as the quantitative determination of echinacoside and β-ecdysone in Bushen Jiannao Grains. The samples were extracted with ethanol and separated on Waters ACQUITY UPC² BEH column (100 mm x 3. 00 mm, 1. 7 μm), with a gradient supercritical CO₂ - 0.05% phosphoric acid-methanol solvent system at 40 ℃. The flow rate was 0.8 mL/min, the detection wavelength was set at 248 nm and the injection volume was 1 μL. Results showed that all the main peaks in the fingerprint were clearly attributed. The peak named 12 was β-ecdysone with the content of 380μg/g and the peak named 15 was echinacoside with the content of 9.562 mg/g. The method was simple, eco-friendly, accurate and reliable compared with HPLC and UPLC.

243. Fast Analysis Of Common Fatty Acids In Edible Vegetable Oils By Ultra-Performance Convergence Chromatography-Mass Spectrometry

Keywords: Fatty Acids; Plant Oils; Vegetables; Chromatography; Mono-unsaturated; Tandem Mass Spectrometry; UPC²

Abstract
A fast analytical method for five common fatty acids in six edible vegetable oils was developed by ultra-performance convergence chromatography-mass spectrometry (UPC2-MS). The five fatty acids are palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. Their contents in the corn oil, sunflower oil, soybean oil, tea oil, rapeseed oil and peanut oil were compared. The chromatographic separation was performed on an ACQUITY UPC² BEH 2-EP column (100 mm x 2.1 mm, 1.7 μm) using the mobile phases of carbon dioxide and methanol/acetonitrile (1:1, v/v) with gradient elution. The separated compounds were detected by negative electrospray ionization ESF-MS. The results showed that the reasonable lineairities were achieved for all the analytes over the range of 0.5-100 mg/L with the correlation coefficients (R²) of 0.9985-0.9998. The limits of quantification (S/N ≥ 10) of the five fatty acids were 0.15-0.50 mg/L. The recoveries of the five fatty acids at three spiked levels were in the range of 89.61%-108.50% with relative standard deviations of 0.69%-3.01%. The developed method showed high performance, good resolution and fast analysis for the underivatized fatty acids. It has been successfully used to detect the five fatty acids from corn oil, sunflower oil, soybean oil, tea oil rapeseed oil and peanut oil.
244. Alkaloids Analysis Using An Off-Line Two-Dimensional Supercritical Fluid Chromatography × Ultra-High Performance Liquid Chromatography

Keywords: East China University, alkaloids, BEH, BEH 2-EP, XAmide, CSH FP, HSS T3, pharmaceutical

Abstract
In this study, an off-line two-dimensional (2-D) supercritical fluid chromatography (SFC) × ultra-high performance liquid chromatography (UHPLC) method with high orthogonality was developed for the analysis of the practical amide alkaloids fraction from *P. longum* L. The effects of SFC parameters such as column type, organic modifier, temperature and back-pressure on separation were systematically evaluated. Different selectivity was observed for different columns (BEH, BEH 2-EP, XAmide and CSH FP). An investigation was then carried out of the orthogonality of different columns and systems following a geometric approach with a set of amide alkaloid samples. The orthogonality between a CSH FP column and a BEH column reached 50.79%, which was much higher than that for the other columns. While the orthogonality between SFC and UHPLC based on an XAmide column and an HSS T3 column reached 69.84%, which was the highest of all the combinations. At last, the practical amide alkaloids fraction was analyzed with an off-line 2-D chromatography SFC × UHPLC system. In total, at least 340 peaks were detected by this method. Rapid separation in these two dimensions and easy post treatment of SFC facilitated this 2-D system for the separation of complex samples.

http://dx.doi.org/10.1039/C4AN00438H

245. Modern Analytical Supercritical Fluid Chromatography Using Columns Packed With Sub-2 Mm Particles: A Tutorial

Keywords: Charles University, University of Geneva, Waters Corporation, lipophilic, hydrophilic, basic drugs, tutorial, pharmaceutical

Abstract
This tutorial provides an overview of the possibilities, limitations and analytical conditions of modern analytical supercritical fluid chromatography (SFC) using columns packed with sub-2 μm particles. In particular, it gives a detailed overview of commercially available modern SFC instrumentation and the detectors that can be employed (UV, MS, ELSD, FID, etc.). Some advice on the choice of the stationary phase dimensions and chemistries, the nature of the mobile phase (choice of organic modifier and additives) and its flow rate as well as the backpressure and temperature are also provided. Finally, several groups of potentially problematic compounds, including lipophilic compounds, hydrophilic substances and basic drugs, are discussed in detail. All these families of analytes can be resolved with SFC but require specific analytical conditions.

http://dx.doi.org/10.1016/j.aca.2014.03.034
246. Analysis Of Paclitaxel In Pharmaceutical Injection By Ultra Performance Convergence Chromatography

Keywords: Paclitaxel, Chinese Academy of Tropical Agricultural Sciences, Shannxi University, chemotherapy, BEH -2EP, impurity profiling, pharmaceutical

Abstract
A simple and sensitive ultra-performance convergence chromatography system coupled with a diode array detector for analysis of paclitaxel, 10-deacetyl-7-epipaclitaxel and cephalomannine in paclitaxel injection was developed. The analyses were carried out on a Waters Acquity UPC2 BEH 2-EP using the mobile phase of mixture of supercritical CO2 and methanol with a linear gradient elution from 92:8 to 82:18 at 50 °C. The method offers good linearity, higher resolution, and good repeatability. LOD and LOQ were 0.45 and 1.32 ng, respectively.

http://dx.doi.org/10.4028/www.scientific.net/AMR.989-994.1060

247. Stereoselective Separation And Pharmacokinetic Dissipation Of The Chiral Neonicotinoid Sulfoxaflor In Soil By Ultraperformance Convergence Chromatography / Tandem Mass Spectrometry

Keywords: Chinese Academy of Agricultural Sciences, sulfoxaflor, stereoisomeric separation, enantioselective degradation, Chiralpak IA-3, soil, environmental

Abstract
Ultraperformance convergence chromatography/tandem triple quadrupole mass spectrometry (UPC²-MS/MS) is a novel tool in separation science that combines the advantages of supercritical fluid chromatography with ultraperformance liquid chromatography/MS/MS technology. The use of nontoxic CO2 fluid and a postcolumn additive to complement MS/MS allows better control of analyte retention for chiral separation and high-sensitivity determination with different chiral stationary phases. This paper reports the stereoselective separation and determination of the chiral neonicotinoid sulfoxaflor in vegetables and soil by UPC²-MS/MS. Baseline resolution (Rs ≥ 1.56) of and high selectivity (LOQ ≤ 1.83 μg/kg) for the four stereoisomers were achieved by postcolumn addition of 1 % formic acid–methanol to a Chiralpak IA-3 using CO2/isopropanol/acetonitrile as the mobile phase at 40 °C, 2,500 psi, and for 6.5 min in electrospray ionization positive mode. Rearranged Van’t Hoff equations afforded the thermodynamic parameters ΔH ° and ΔS °, which were analyzed to promote understanding of the enthalpy-driven separation of sulfoxaflor stereoisomers. The interday mean recovery, intraday repeatability, and interday reproducibility varied from 72.9 to 103.7 %, from 1.8 to 9.2 %, and from 3.1 to 9.4 %, respectively. The proposed method was used to study the pharmacokinetic dissipation of sulfoxaflor stereoisomers in soil under greenhouse conditions. The estimated half-life ranged from 5.59 to 6.03 d, and statistically nonsignificant enantioselective degradation was observed. This study not only demonstrates that the UPC²-MS/MS system is an efficient and sensitive method for sulfoxaflor stereoseparation, but also provides the first experimental evidence of the pharmacokinetic dissipation of sulfoxaflor stereoisomers in the environment.

http://dx.doi.org/10.1007/s00216-014-8089-9
248. Investigating Sub-2 μm Particle Stationary Phase Supercritical Fluid Chromatography Coupled To Mass Spectrometry For Chemical Profiling Of Chamomile Extracts

Keywords: Waters, University of Mississippi, Kings College, chamomile, tea extracts, food

Abstract

Roman and German chamomile are widely used throughout the world. Chamomiles contain a wide variety of active constituents including sesquiterpene lactones. Various extraction techniques were performed on these two types of chamomile. A packed-column supercritical fluid chromatography-mass spectrometry method was designed for the identification of sesquiterpenes and other constituents from chamomile extracts with no derivatization step prior to analysis. Mass spectrometry detection was achieved by using electrospray ionization. All of the compounds of interest were separated within 15 min. The chamomile extracts were analyzed and compared for similarities and distinct differences. Multivariate statistical analysis including principal component analysis and orthogonal partial least squares-discriminant analysis (OPLS-DA) were used to differentiate between the chamomile samples. German chamomile samples confirmed the presence of cis- and trans-tonghaosu, chrysosplenols, apigenin diglucoside whereas Roman chamomile samples confirmed the presence of apigenin, nobilin, 1,10-epioxynobilin, and hydroxyisotonobilin.

http://dx.doi.org/10.1016/j.aca.2014.06.031

249. Chromatographic Evidence Of Silyl Ether Formation (SEF) In Supercritical Fluid Chromatography

Keywords: Waters Corporation, silyl ether formation, surface chemistry, particle chemistry, separation science

Abstract

In this article, we propose that silyl ether formation (SEF) is a major contribution to retention and selectivity variation over time for supercritical fluid chromatography (SFC). In the past, the variations were attributed to instrumentation, but high performance SFC systems have shed new light on the source of variation. As silyl ethers form on the particle surface, the hydrophilicity is decreased, significantly altering the retention and selectivity observed. SEF is expected to occur with any chromatographic particle containing silanols but is slowed on hybrid inorganic/organic particles. The SEF reaction is between alcohols on the particle surface and in the mobile phase solvent. We have found that storage conditions of a column are paramount, which can either prevent or accelerate the process. Because SEF exists as an equilibrium between the liquid phase and the particle surface, the process is also reversible. The silanols can be hydroxylated (regenerated) to their original state upon exposure to water. The next generation of stationary phases will either advantageously utilize SEF or effectively mitigate its effects. Mitigation of SEF would be a significant improvement in SFC that has the potential to vault their performance to levels of similar reproducibility and reliability observed for high performance liquid
chromatography (HPLC). Further research in SEF may lead to a better understanding of the mechanism of interaction between the solutes and chromatographic surface.

http://dx.doi.org/10.1021/ac5035709

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**250. Supercritical Fluid Chromatography As A Tool For Enantioselective Separation – A Review**

2014 - Analytica Chimica Acta

Keywords: Charles University, enantioselective separation, enantiomers, chiral separations, review

**Abstract**

Supercritical fluid chromatography (SFC) has become popular in the field of enantioselective separations. Many works have been reported during the last years. This review covers the period from 2000 till August 2013. The article is divided into three main chapters. The first one comprises a basic introduction to SFC. The authors provide a brief explanation of general principles and possibilities of this method. The advantages and drawbacks are also listed. Next part deals with chiral separation systems available in SFC, namely with the commonly used chiral stationary phases. Properties and interaction possibilities of the chiral separation systems are described. Recent theoretical papers are emphasized in this chapter. The last part of the paper gives an overview of applications of enantioselective SFC in analytical chemistry, in both analytical and preparative scales. Separation systems and conditions are summed up in tables so that they provide a helpful tool for analysts who search for a particular method of analysis.

http://dx.doi.org/10.1016/j.aca.2014.02.036

Abstract
Triacylglycerol (TAG) as the major ingredient of oil is a key indicator for the identification of oil adulteration due to its characteristic distributions in different oils. In this research, a high-throughput method for rapidly detecting acylglycerols in various edible oils was established by applying supercritical fluid chromatography (SFC) coupled with quadruple time-of-flight mass spectrometry (Q-TOF-MS), without any sample pretreatment procedure. The retention mechanism of TAGs on different columns were comprehensively evaluated including the previous work on an ODS column, as well as our study on HSS C_{18}SB, BEH and BEH 2-EP columns. Distinctive retention of our method allows the separation of certain pairs of TAGs which remains difficult by traditional methods, and the analysis time is greatly reduced. SFC-MS data were subsequently analysed by principal component analysis (PCA) to make a clear classification between six different kinds of vegetable oils, showing its potential in differentiating the fakes quickly. Considering the high-price of olive oil, further quantification of olive oil adulteration was studied by estimating known and unknown compositions of blend oils according to the curves of areas of selected TAG markers versus adulterant concentrations. Good consistencies with the labeled content of olive oil in commercial blend oils demonstrated the reliability of the quantitative method, and these blend oils were differentiated distinctly from pure olive oils in the score plot of a PCA model. To the best of our knowledge, it was the first time that the retention of TAGs using different stationary phases in a SFC system was discussed, and that SFC was applied to quantify oil adulteration. This fast and effective method is of great advantage to authenticate edible oils.

http://dx.doi.org/10.1039/C6AY00970K

252. Rapid Analysis Of Non-Steroidal Anti-Inflammatory Drugs In Tap Water And Drinks By Ionic Liquid Dispersive Liquid–Liquid Microextraction Coupled To Ultra-High Performance Supercritical Fluid Chromatography

Abstract
A novel rapid analytical method for the determination of four non-steroidal anti-inflammatory drugs (NSAIDs) – nabumetone, ibuprofen, naproxen and diclofenac, NSAIDs, environmental

Keywords: Beijing University of Chemical Technology, tap water, drinking water, non-steroidal anti-inflammatory drugs, nabumetone, ibuprofen, naproxen and diclofenac, NSAIDs, environmental
strategies to screen and optimize the experimental variables such as the volume of the ionic liquid, the volume of the disperser solvent, sample pH, ionic strength, ultrasonication time and centrifugation time which affected the extraction efficiency. Separation conditions of UHPSFC, such as column screening, modifiers, column temperature, back pressure and flow rate, were also optimized in this study. 4 NSAIDs were simultaneously separated and determined in 2.1 minutes. The optimized US-IL-DLLME-UHPSFC-PDA method showed good enrichment factors (126–132) and recoveries (81.37–107.47%) for the rapid extraction of nabumetone, ibuprofen, naproxen and diclofenac in tap water and drinks. The method’s limits of detection for nabumetone, ibuprofen, naproxen and diclofenac were 1.56, 7.69, 0.62, and 7.37 ng mL$^{-1}$ with excellent linearity ($R > 0.9957$).

http://dx.doi.org/10.1039/c4ay01305k

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253. Analysis Of Chemical Constituents In Jackfruit Peel By UPC$^2$ / Q-TOF-MS Method

2014 - Applied Mechanics and Materials

Keywords: Chinese Academy of Tropical Agricultural Sciences, Northeast Agricultural University, Hainan University, jackfruit peel, HSS, food

Abstract

A new method for the analysis chemical constituents in jackfruit peel by ultra performance convergence chromatography (UPC$^2$) was developed for the first time. The extraction of jackfruit peel was carried out under classical heating with ultrasonic wave and extracted with ethyl ether. The analytes were separated on an ACQUITY UPC$^2$ HSS C18 SB column with a gradient elution (80:20 to 99.8:0.2) of mobile phase consisting of CO$_2$ and methanol. The result indicated that UPC$^2$/Q-TOF-MS is a simple, rapid, reliable and effective method to analyze the biochemical compounds in jackfruit peel. A total of 65 compounds were identified, including acids, esters, alcohols, and pyrazine, etc.

http://dx.doi.org/10.4028/www.scientific.net/AMM.556-562.607

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254. Organocatalytic Cascade Reactions: Diversity-Oriented Synthesis For The Construction Of Hydroisoquinoline Scaffolds

2014 - Chemical Communications

Keywords: Aarhus University, enantioselective synthesis, hydroisoquinolines, separation science

Abstract

Novel hydrophobized hyaluronan (HA) derivatives, containing ω-phenylalkanoic acids (ω-PAA, 4-phenylbutyric acid, 6-phenylhexanoic, 8-phenyloctanoic or 11-tolylundecanoic acids) were prepared by esterification. Mixed anhydrides obtained after reaction of the carboxyl acid moiety and benzoyl chloride were found to be active acylating agents, affording hydrophobized HA in good yield and under mild conditions. The reactivity of the aromatic fatty acids towards esterification has decreased with the increasing length of the aliphatic spacer between the aromatic substituent and carboxylic acid moiety. The novel HA derivatives self-assembled from very low concentrations and were found to be non-cytotoxic. The potential use of ω-phenylalkanoic acids grafted-HA towards drug delivery applications was demonstrated by hydrophobic drugs (resveratrol and retinyl palmitate) encapsulation. The drug loading capacity of the novel HA derivatives was
significantly improved most likely because of n–n interactions between the micelle core and loaded hydrophobic aromatic compound.
http://dx.doi.org/10.1039/C4CC01231C

255. Organocatalytic Cascade Reactions: Towards The Diversification Of Hydroisochromenes And Chromenes Through Two Different Activation Modes

Keywords: Aarhus University, hydroisochromenes, chromenese, organocatalysis, separation science

Abstract
The organocatalytic enantioselective syntheses of functionalized hydroisochromenes and chromenes by trienamine-mediated [4+2]-cycloaddition/nucleophilic ring-closing and iminium/aminal-mediated oxa-Michael/Michael/nucleophilic ring-closing with 2-nitroallylic alcohols are presented. The corresponding cycloadducts, with up to five stereocenters, are formed in good yield and excellent enantioselectivities. The synthetic applications of the obtained products have been demonstrated.
http://dx.doi.org/10.1002/chem.201403505

256. Fast Separation Method Development For Supercritical Fluid Chromatography Using An Autoblending Protocol

Keywords: Chengdu Institute of Biology, method development, enantioseparations, health science

Abstract
Supercritical fluid chromatography (SFC) is a powerful separation technique particularly in the area of enantioseparations. With rapid analysis speed, wide polarity compatibility, higher column efficiency and lower cost of the mobile phase, SFC is regarded as a better choice than high-performance liquid chromatography for drug discovery. In the development of separation method, the choice of modifier and/or additive is the key point of optimum separation. However, such screening of SFC is typically time-consuming. In this study, an autoblending protocol was introduced to speed up the modifier and/or additive screening process, which was performed on a separate programmable gradient proportioning system. The protocol prepares mobile phases on the fly to speed up the screening of modifiers and/or additives and reduces the waste of solutions. Furthermore, by switching mobile phase in the same run, separation of different types of compounds could also be achieved. This system was successfully applied to screen modifier–additive combinations of three alkaloids and three polyphenols by switching to two mobile phase conditions, as well as by a ternary additive mobile phase on an SFC system. The proposed protocol allows fast separation method development of SFC, which was proved to be rapid, simple, and reproducible.
http://dx.doi.org/10.1007/s10337-014-2684-y

257. Separation Of Pharmaceuticals By SFC Using Mono And Di-Hydroxy Substituted Phenyl Stationary Phases

Keywords: Pfizer Global Research, sfc stationary phases, pharmaceutical
Abstract
Supercritical Fluid Chromatography (SFC) has gained significant momentum over the past 10 years as the technique of choice for analytical and preparative separation of small organic molecules. One of the main contributors to its success is the selectivity enhancements derived from multifunctional stationary phases above and beyond that achieved through mobile phase tuning. While the pyridine column has become a standard column in SFC by expanding the range of compounds amenable to SFC, the monofunctional pyridine phase may be too polar to be broadly applicable. Likewise, the C18 and phenyl columns may be too non-polar for many compounds. Therefore, in order to offer enhanced selectivity in SFC, these phases are often substituted. For instance, adding polar functional groups to the pyridine phase have produced alternatives phases such as hydroxylamino dipyridinyl column. In a similar approach, more polar functional groups can be added to the phenyl phase to increase its overall polarity and thus improve its selectivity and applicability. In this application note, we highlight selectivity changes observed when introducing mono- and di-hydroxy polar groups onto phenyl phases and comparing them to their unsubstituted counterpart. 

258.Delivering The Promise Of SFC: A Case Study

Keywords: AstraZeneca, purification, case study, pharmaceutical

Abstract
During the past years there has been a rapid development in supercritical fluid chromatography (SFC) instrumentation making it a highly efficient and robust technique. Although much is written about the advantages of SFC over liquid chromatography (LC), there are not many direct comparisons detailing the gain in purification throughput, the savings in solvent consumption and the reduced environmental impact for large-scale SFC applications. We will show that a research scale separation laboratory built to handle multigram amounts can be used for kilogram separations when moving from LC to SFC. 
http://dx.doi.org/10.1016/j.drudis.2014.04.021

259.A New Method For Determination Of A-Tocopherol In Tropical Fruits By Ultra Performance Convergence Chromatography With Diode Array Detector

Keywords: Agricultural Product Processing Research Institute, Chinese Academy of Tropical Agricultural Sciences, tocopherol, food

Abstract
A new method for the analysis of α-tocopherol in tropical fruits by ultra performance convergence chromatography (UPC²) was developed for the first time. Five varieties of tropical fruit samples were separately saponified under classical heating and extracted with ether. The extracted α-tocopherol was separated on a BEH column, with a mobile phase consisting of CO₂ and methanol, with a gradient elution (99:1 to 90:10), and detected with diode array detector at 293 nm. The limit of detection (LOD) and limit of quantification (LOQ) were about 60.0 and 103.3 ng, respectively. This method was considered to be simple, fast and reliable, and successfully applied to analysis of α-tocopherol in tropical fruits. The values of α-tocopherol in pitaya, jackfruit, durians, mango, and papaya ranged from 0.16 to 0.45 mg/100 g dry weight in edible portion. Recovery rates obtained by the standard addition method on these tropical fruit samples ranged from 95.4 to 101.4 % with high repeatability (RSD, 1.2–2.6 %). 
http://dx.doi.org/10.1007/s12161-014-9789-7

260.Ultra-Performance Convergence Chromatography (UPC²) Method For The Analysis Of Biogenic Amines In Fermented Foods

Keywords: Agricultural Product Processing Research Institute, Chinese Academy of Tropical Agricultural Sciences, biogenic amines, fermented foods, yulu, sufu, food

Abstract
A rapid ultra-performance convergence chromatography (UPC(2)) method for the determination of eight biogenic amines (spermine, spermidine, putrescine, cadaverine, tryptamine, phenylethylamine, histamine and tyramine) in fermented foods was developed. The amines were pre-column derivatized with dansyl chloride and separated on UPC(2) system with a HSS C18 SB column (3.0 × 100 mm, 1.8 μm) using gradient elution with a binary system of CO2 and n-hexane:isopropanol:ammonium hydroxide (70:30:0.15, v/v/v), back pressure of 2,000 psi, flow rate of 2.0 ml/min and DAD detection at 254 nm. The result showed excellent linearity (r=0.9995-1.0000). Limits of detection (LOD) and limits of quantification (LOQ) were 21-67 ng/L and 72-224 ng/L, respectively. Relative standard deviations (RSD) for repeatability and reproducibility were 0.21-0.87% and 1.98-4.02%, respectively. Furthermore, this method was successfully applied to analysis of biogenic amines in Yulu and Sufu samples.

http://dx.doi.org/10.1016/j.foodchem.2014.04.063

261. Chemical Profiling Of Triacylglycerols And Diacylglycerols In Cow Milk Fat By Ultra-Performance Convergence Chromatography Combined With Quadrupole Time-Of-Flight Mass Spectrometry

Keywords: Shanghai Jiao Tong University, triacylglycerols and diacylglycerols tags, dags, cow milk, food

Abstract
An ultra-performance convergence chromatography (UPC(2)) system coupled with quadrupole time-of-flight mass spectrometry (Q-TOF-MS) was successfully utilised to analyse triacylglycerols and diacylglycerols in cow milk fat. This novel approach obtained an improved resolution of triacylglycerols in comparison to previously reported chromatographic methods combined with MS detector in a shorter analytical time. A total of 49 triacylglycerols and 7 diacylglycerols were identified according to their secondary MS profiles and elementary composition. Furthermore, UPC(2) is an environmental friendly analytical method with a drastic reduction of organic solvent usage. The established UPC(2)-MS approach has potential application in lipidomics as an alternative method besides LC-MS and GC-MS.

http://dx.doi.org/10.1016/j.foodchem.2013.07.114

262. Preparation Of Bio-Based Surfactants From Glycerol And Dodecanol By Direct Etherification

Keywords: East China University of Science and Technology, surfactants, glycerol, dodecanol, chemical materials

Abstract
In this paper, we studied an original synthetic strategy to prepare bio-based surfactants by direct solvent-free etherification of glycerol with dodecanol using heterogeneous interfacial acidic catalysts dubbed Pickering Interfacial Catalysis. The conversion of dodecanol could be achieved to 60–71% with limited production of didodecyl ether (DE) as the main side product. The selectivity of the final product, alkylpolyglycerylether (AGEM), could be pushed to >80% with a water removal process at 150 °C, as a mixture of monolauryl polyglyceryl ethers, multilauryl polyglyceryl ethers and multilauryl cyclicpolyglyceryl ethers. AGEM could be isolated with a suitable work-up and was fully characterized by GC (MS), HPLC, SFC/HRMS, etc. The physicochemical properties of these new surfactants were evaluated, as well as their laundry performances. This solvent-free direct etherification process paves the way towards new value-added applications of glycerol.

http://dx.doi.org/10.1039/C4GC00818A

263. Green And Sensitive Supercritical Fluid Chromatographic–Tandem Mass Spectrometric Method For The Separation And Determination Of Flutriafol Enantiomers In Vegetables, Fruits, And Soil

2014 - Journal of Agricultural and Food Chemistry
Abstract
A green and sensitive chiral analytical method was developed to determine flutriafol enantiomers in vegetables (tomato, cucumber), fruits (apple, grape), and soil by supercritical fluid chromatography–tandem mass spectrometry. The enantioseparation was performed within 3.50 min using Chiralpak IA-3 column with CO2/methanol (88:12, v/v) as the mobile phase at a 2.2 mL/min flow rate. The postcolumn compensation technology provided with 1% formic acid/methanol greatly improved the ionization efficiency of mass spectrometry. Column temperature, auto back pressure regulator pressure, and flow rate of compensation solvent were optimized to 30 °C, 2200 psi, and 0.1 mL/min, respectively. The simple and fast QuEChERS pretreatment method was adopted. Mean recoveries for flutriafol enantiomers were 77.2–98.9% with RSDs ≤ 9.6% in all matrices. The limits of quantification ranged from 0.41 to 1.18 μg/kg. Well-applied to analyze authentic samples, the developed method could act as a versatile strategy for the analysis of flutriafol enantiomers in food and environmental matrices.
http://dx.doi.org/10.1021/jf504324t

264. Catalytic Asymmetric Synthesis Of 4-Nitropyrazolidines: An Access To Optically Active 1,2,3-Triamines
2014 - Journal of the American Chemical Society

Abstract
The first catalytic enantio- and diastereoselective synthesis of 4-nitropyrazolidines is presented. Asymmetric hydrogen-bonding activation of nitro-olefins facilitated the 1,3-dipolar cycloaddition with hydrazones, affording optically active 4-nitropyrazolidines containing three continuous stereogenic centers as a single diastereomer in up to 99% ee. Furthermore, it is demonstrated that the optically active 4-nitropyrazolidines can be applied as precursors for the synthesis of highly interesting 1,2,3-triamines.
http://dx.doi.org/10.1021/ja506694v
265. Evaluation Of Co-Solvent Fraction, Pressure And Temperature Effects In Analytical And Preparative Supercritical Fluid Chromatography

Keywords: Karlstadt University, chemometrics, design of experiments, separation science

Abstract
A chemometric approach is used for studying the combined effect of temperature, pressure and co-solvent fraction in analytical and preparative supercritical fluid chromatography (SFC). More specifically, by utilizing design of experiments coupled with careful measurements of the experimental conditions the interaction between pressure, temperature and co-solvent fraction was studied with respect to productivity, selectivity and retention in chiral SFC. A tris-(3,5-dimethylphenyl) carbamoyl cellulose stationary phase with carbon dioxide/methanol as mobile phase and the two racemic analytes trans-stilbene oxide (TSO) and 1,1′-bi-2-naphthol (BINOL) were investigated. It was found for the investigated model system that the co-solvent fraction and pressure were the parameters that most affected the retention factors and that the co-solvent fraction and column temperature were most important for controlling the selectivity. The productivity in the preparative mode of SFC was most influenced by the co-solvent fraction and temperature. Both high co-solvent fraction and temperature gave maximum productivity in the studied design space.

http://dx.doi.org/10.1016/j.chroma.2014.11.045

266. Comparison Of Liquid Chromatography And Supercritical Fluid Chromatography Coupled To Compact Single Quadrupole Mass Spectrometer For Targeted In Vitro Metabolism Assay

Keywords: University of Geneva, method development, in vitro metabolism, column screening, phytochemical inhibitors, pharmaceutical

Abstract
The goal of this study was to evaluate the combination of powerful chromatographic methods and compact single quadrupole MS device for simple in vitro cytochrome P450 (CYP) inhibition assay, instead of more expensive triple quadrupole MS/MS detectors. For this purpose, two modern chromatographic approaches (ultra-high pressure liquid chromatography (UHPLC) and ultra-high performance supercritical fluid chromatography (UHPSFC)) were tested in combination with simple MS detector. To show the applicability for an in vitro CYP-mediated metabolism assay using the cocktail approach, a method was first developed in UHPLC-MS to separate a mixture of 8 probe substrates and 8 CYP-specific metabolites. A screening procedure was initially applied to determine the best combination of a column, an organic modifier and a mobile-phase pH, followed by fine tuning of the conditions (i.e., gradient profile, temperature and pH) using HPLC modelling software. A similar sequential method development procedure was also evaluated for UHPSFC-MS. For method development, where peak tracking is necessary, the use of single quadrupole MS was found to be extremely valuable for following the investigated analytes. Ultimately, a baseline separation of the 16 compounds was achieved in both UHPLC-MS and UHPSFC-MS with an analysis time of less than 7 min. In a second series of experiments, sensitivity was evaluated, and LOQ values were between 2 and 100 ng/mL in UHPLC-MS, while they ranged from 2 to 200 ng/mL in UHPSFC-MS. Based on the concentrations employed for the current in vitro phase I metabolism assay, these LOQ values were appropriate for this type of application. Finally, the two analytical methods were applied to in vitro CYP-dependent metabolism testing. Two well-known
phytochemical inhibitors, yohimbine and resveratrol, were investigated, and reliable conclusions were drawn from these experiments with both UHPLC-MS and UHPSFC-MS. At the end, the proposed strategy of optimized chromatography combined with simple MS device has been shown to potentially compete with the widely used combination of generic chromatography and highly selective MS/MS device for simple in vitro CYP inhibition assays. In addition, our analytical method may be easier to use in a routine environment; the instrument cost is significantly reduced and the two developed methods fit for purpose.

http://dx.doi.org/10.1016/j.chroma.2014.10.055

267. Evolution Of Strategies To Achieve Baseline Separation Of Ten Anionic, Water-Soluble Sulfated Estrogens Via Achiral Packed Column Supercritical Fluid Chromatography

Keywords: Pfizer, Waters, Virginia Tech, 2-ethylpyridine, sulfated estrogen salts, health science

Abstract
Near baseline separation of ten sulfated sodium salts of various structurally related estrogens employing a variety of bonded stationary phase packed columns was obtained using a conventional supercritical fluid chromatograph coupled with UV detection. Critical pairs 2/3 (8,9-dehydroestrone/17β-dihydroequilin) and 6/7 (17α-estradiol or 17α-dihydroequilin/estrone), however, failed to baseline separate. In all preliminary separations, 10mM ammonium acetate and variable percentages of H2O were initially used as co-additives in conjunction with methanol as a modifier. Different modifier programs and temperatures were employed to optimize the separation in a timely manner. A 2-ethylpyridine column provided the best separation compared to bare silica, diol, and cyano-based bonded phase columns. The employment of both salt and water as additives to the methanol-modified CO2 mobile phase suggested a mixed mode separation mechanism involving both ion pairing of each anionic sulfated estrogen with ammonium ion and hydrophilic interaction facilitated by partitioning of analyte between the aqueous solvated stationary phase and the aqueous component of the mobile phase. Upon more extensive study with either iso-propylamine or formic acid-ammonium formate buffer, the critical anionic pairs were 95% baseline resolved.

http://dx.doi.org/10.1016/j.chroma.2014.10.021

268. Evaluation Of The Quantitative Performances Of Supercritical Fluid Chromatography: From Method Development To Validation

Keywords: University of Liege, quantitative performance, design space, pharmaceutical quality control, drugs, method validation

Abstract
Recently, the number of papers about SFC increased drastically but scientists did not truly focus their work on quantitative performances of this technique. In order to prove the potential of UHPSFC, the present work discussed about the different steps of the analytical life cycle of a method: from development to validation and application. Moreover, the UHPSFC quantitative performances were evaluated in comparison with UHPLC, which is the main technique used for quality control in the pharmaceutical industry and then could be considered as a reference. The methods were developed using Design Space strategy, leading to the optimization of robust method. In this context, when the Design Space optimization shows guarantee of quality, no more robustness study is required prior to the validation. Then, the methods were geometrically transferred in order to reduce the analysis time. The UHPSFC and UHPLC methods were validated based on the total error approach using accuracy profile. Even if UHPLC showed better precision and sensitivity, UHPSFC method is able to give accurate results in a dosing range larger than the 80-120% range required by the European Medicines Agency. Consequently, UHPSFC results are valid and could be used for the control of active substance in a finished pharmaceutical product. Finally, UHPSFC validated method was used to analyse real samples and gave similar results than the reference method (UHPLC).

http://dx.doi.org/10.1016/j.chroma.2014.01.046
269. A Scaling Rule In Supercritical Fluid Chromatography. I. Theory For Isocratic Systems

Keywords: Waters, method transfer, pressure drop, density variation, density modulation, preparative, scaling, scale-up, separation science

Abstract
Scaling is regularly done in chromatography either to transfer a successfully designed method of analysis developed in one system to another system, or to scale-up a separation method developed in analytical scale to preparative scale. For liquid chromatography there are well-tested guidelines for scaling, which makes it a routine job. For supercritical fluid chromatography (SFC), on the other hand, neither do we have any well-understood principles behind scaling nor do we know how far the strategies applied in LC could be applicable to SFC. In this article, we have addressed these issues and proposed a rule applicable for scaling isocratic methods between different SFC systems and column dimensions under commonly used operating temperatures and pressures. We have shown that the scale-up and method transfer techniques used in LC can be applied to SFC, provided we ensure that both the original and the target systems in SFC operate at the same average density. The current article will present the theory, discuss the extents of applicability of this rule, and outline its limitations. In an accompanying article implementation of this rule in various practical situations will be presented.

http://dx.doi.org/doi:10.1016/j.chroma.2014.08.009

270. Simultaneous Analysis For Water- And Fat-Soluble Vitamins By A Novel Single Chromatography Technique Unifying Supercritical Fluid Chromatography And Liquid Chromatography

Keywords: Osaka University, fat soluble vitamins, water soluble vitamins, food

Abstract
Chromatography techniques usually use a single state in the mobile phase, such as liquid, gas, or supercritical fluid. Chromatographers manage one of these techniques for their purpose but are sometimes required to use multiple methods, or even worse, multiple techniques when the target compounds have a wide range of chemical properties. To overcome this challenge, we developed a single method covering a diverse compound range by means of a "unified" chromatography which completely bridges supercritical fluid chromatography and liquid chromatography. In our method, the phase state was continuously changed in the following order; supercritical, subcritical and liquid. Moreover, the gradient of the mobile phase starting at almost 100% CO2 was replaced with 100% methanol at the end completely. As a result, this approach achieved further extension of the polarity range of the mobile phase in a single run, and successfully enabled the simultaneous analysis of fat- and water-soluble vitamins with a wide logP range of -2.11 to 10.12. Furthermore, the 17 vitamins were exceptionally separated in 4min. Our results indicated that the use of dense CO2 and the replacement of CO2 by methanol are practical approaches in unified chromatography covering diverse compounds. Additionally, this is a first report to apply the novel approach to unified chromatography, and can open another door for diverse compound analysis in a single chromatographic technique with single injection, single column and single system.

http://dx.doi.org/doi:10.1016/j.chroma.2014.08.003

271. Evaluation Of Stationary Phases Packed With Superficially Porous Particles For The Analysis Of Pharmaceutical Compounds Using Supercritical Fluid Chromatography

Keywords: Osaka University, fat soluble vitamins, water soluble vitamins, food

Abstract
Superficially porous particles (SPP), or core shell particles, which consist of a non-porous silica core surrounded by a thin shell of porous silica, have gained popularity as a solid support for chromatography over the last decade. In the present study, five unbonded silica, one diol, and two ethylpyridine (2-ethyl and 4-ethyl) SPP columns were evaluated under SFC conditions using two mixtures, one with 17 drug-like compounds and the other one with 7 drug-like basic compounds. Three of the SPP phases, SunShell™ 2-ethylpyridine (2-EP), Poroshell™ HILIC, and Ascentis®
Express HILIC, exhibited superior performances relative to the others (reduced theoretical plate height (hmin) values of 1.9–2.5 for neutral compounds). When accounting for both achievable plate count and permeability of the support using kinetic plot evaluation, the Cortecs™ HILIC 1.6 μm and Ascentis® Express HILIC 2.7 μm phases were found to be the best choices among tested SPPs to reach efficiencies up to 30,000 plates in the minimum amount of time. For desired efficiencies ranging from 30,000 to 60,000 plates, the SunShell™ 2-EP 2.6 μm column clearly outperformed all other SPPs. With the addition of a mobile phase additive such as 10 mM ammonium formate, which was required to elute the basic components with sharp peaks, the Poroshell™ HILIC, SunShell™ Diol and SunShell™ 2-EP phases represent the most orthogonal SPP columns with the highest peak capacities. This study demonstrates the obvious benefits of using columns packed with SPP on current SFC instrumentation.

http://dx.doi.org/doi:10.1016/j.chroma.2014.07.078

272. Effect Of Particle Size On The Speed And Resolution Of Chiral Separations Using Supercritical Fluid Chromatography

Keywords: Merck, Celgene, Chiralcel, pharmaceutical

Abstract
Fast chiral supercritical fluid chromatography (SFC) separations have become important due to the increasing use of high-throughput experimentation (HTE) in organic synthesis. These HTE experiments can generate hundreds of samples for chiral analysis that need to be assayed in a short time. In general, chiral SFC can provide much faster analysis times compared to liquid chromatography (LC). Additionally, columns packed with smaller particles can provide faster and more efficient separations. In this study, the effect of the particle size on the speed and resolution of chiral separations by SFC was evaluated. The performance of Chiralcel OD columns packed with either 5 or 3 μm particles were compared using van Deemter or other kinetic plots. The benefits of using smaller particle columns for chiral SFC analysis are illustrated.

http://dx.doi.org/10.1016/j.chroma.2014.07.010

273. Enantioselective High Performance Liquid Chromatography And Supercritical Fluid Chromatography Separation Of Spirocyclic Terpenoid Flavor Compounds

Keywords: Sanofi, University of Hamburg, chiral separations, chiral stationary phases, allylic oxidation, flavors, natural products, terpenes, food

Abstract
Chiral spirocyclic terpenoids are abundant natural flavors with significant impact particularly on the food industry. Chromatographic methods for analytical and preparative separation of these compounds are therefore of high interest to natural product chemists in academia and industry. Gas chromatography on chiral stationary phases is currently the standard method for the separation of volatile terpenoids, limiting the scale to analytical quantities. We report herein high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) protocols for the chiral separation of several racemic spirocyclic terpenoids such as the important flavors theaspirane and vitispirane. A screening of mobile phases and 16 commercially available chiral stationary phases (CSPs) largely based on polysaccharides led to identification of protocols for the separation of all terpenoids tested. SFC methods were found to be particularly useful for the separation of these spirocyclic flavors due to the volatility and low polarity of the compounds. The reported chiral HPLC and SFC protocols are scalable alternatives to gas chromatographic separations of volatile terpenoid flavors.

http://dx.doi.org/10.1016/j.chroma.2014.07.001

274. Insights Into Chiral Recognition Mechanism In Supercritical Fluid Chromatography III. Non-Halogenated Polysaccharide Stationary Phases

Keywords: University of Orleans, Bristol Myers Squibb, chiral recognition, linear solvation energy relationship, polysaccharide, retention relationships, separation science

Abstract
The majority of published enantiomeric separations by supercritical fluid chromatography (SFC) utilize chiral stationary phases (CSP) based on chemically derivatized amylose or cellulose, coated...
or immobilized on silica. There is a large diversity among these polysaccharide-type CSP enhancing the scope of chiral separation applications. But on the other hand, identifying the appropriate support for a given separation problem is rather difficult. Hence, this study aims to provide insights on the difference and similarity among the non-halogenated polysaccharide CSP in terms of retention and selectivity at a molecular level. Firstly, the potential of the clones provided by different manufacturers is evaluated with carbon dioxide - methanol mobile phases. Then different aspects of the chiral recognition mechanism contributing to the separations on 16 different columns of five distinct chiral selectors will be explored based on a large amount of experimental data acquired with the help of modelling and chemometric techniques. We report the influence of the ligand bonded to the polysaccharide on the non-enantio-specific interactions between the solute and the CSP, comparing phenylcarbamate to 3,5-dimethylphenylcarbamate, and 4-methylphénylester to 3,5-dimethylphénylcarbamate. In addition, we evaluate the impact of the silica treatment on the quality of the separation. The phases are characterized in terms of their retention characteristics assessed by the solvation parameter model and separation capabilities assessed by discriminant analysis. 

http://dx.doi.org/10.1016/j.chroma.2014.06.084

275. Insights Into Chiral Recognition Mechanism In Supercritical Fluid Chromatography IV. Chlorinated Polysaccharide Stationary Phases

Keywords: University of Orleans, Bristol Myers Squibb, chemometrics, chlorinated polysaccharides, chiral recognition, linear solvation energy relationship, polysaccharide, retention relationships, separation science

Abstract
The chiral recognition mechanism for a successful enantioseparation on polysaccharide stationary phases are still poorly understood. In this series of papers, we aim to provide some insight into the retention and separation mechanisms occurring in enantioselective supercritical fluid chromatography (SFC). This paper presents a thorough investigation on chlorinated polysaccharide chiral stationary phases (CSP) comprising five coated and three immobilized phases from different manufacturers. The columns are also compared to four non-chlorinated phases to unravel the most significant differences brought about by the introduction of electron-withdrawing atoms on the aromatic ligands. Chemometrics are used to (i) get an overview of all columns (cluster analysis), (ii) describe retention (modified solvation parameter model) and (iii) describe enantioseparation (discriminant analysis). Sample applications are provided to support the discussion.

http://dx.doi.org/10.1016/j.chroma.2014.06.026

276. Generic Chiral Method Development In Supercritical Fluid Chromatography And Ultra-Performance Supercritical Fluid Chromatography

Keywords: Vrije Universiteit Brussel, Bristol Myers Squibb, method development, chiral separations, polysaccharide-based stationary phases, separation science

Abstract
The development of chiral separation methods in pharmaceutical industry is often a very tedious, labour intensive and expensive process. A trial-and-error approach remains frequently used, given the unpredictable nature of enantioselectivity. To speed-up this process and to maximize the efficiency of method development, a generic chiral separation strategy for SFC is proposed in this study. To define such strategy, the effect of different chromatographic parameters on the enantioselectivity is investigated and evaluated. Subsequently, optimization steps are defined to improve a chiral separation in terms of resolution, analysis time, etc. or to induce separation when initially not obtained. The defined strategy proved its applicability and efficiency with the successful separation of a novel 20-compound test set. In a second stage, the method transfer from a conventional to an ultra-performance SFC system is investigated for the screening step of the separation strategy. The method transfer proved to be very easy and straightforward. Similar enantioresolution values, but slightly shorter analysis times were obtained on the ultra-performance equipment. Nevertheless, even more benefit may be expected in ultra-performance SFC when customized sub-2 μm chiral stationary phases will become available.

http://dx.doi.org/10.1016/j.chroma.2014.06.011
277. Determination Of The Average Volumetric Flow Rate In Supercritical Fluid Chromatography

Keywords: University of Tennesse, average volumetric flow rate, nitrous oxide, hold-up volume, separation science

Abstract
This work reviews and discusses controversies and errors made in the determination of the average volumetric flow rate of a compressible mobile phase forced to flow through a chromatographic column. Proper estimates of the volumetric flow rate, which obviously changes along the column, are keys to understanding the retention mechanism that takes place inside the column and to achieve repeatable and reproducible separations. Each step of the calculation process will be discussed in detail, including how to estimate the variations of the pressure and the temperature along the column. The determination of the average volumetric flow rate requires the knowledge of the average density of the mobile phase and of its mass flow rate. The calculations were carried out under various experimental conditions, including different column temperatures and inlet pressures. The estimated values of the volumetric flow rate are validated by the conversion of the retention times to the retention volumes of nitrous oxide peaks, which is valid since this compound is assumed to be non retained, which makes it a hold-up time marker.

http://dx.doi.org/10.1016/j.chroma.2014.02.078

278. Coupling State-Of-The-Art Supercritical Fluid Chromatography And Mass Spectrometry: From Hyphenation Interface Optimization To High-Sensitivity Analysis Of Pharmaceutical Compounds

Keywords: University of Geneva, interfacing approach, detection sensitivity, pharmaceutical

Abstract
The recent market release of a new generation of supercritical fluid chromatography (SFC) instruments compatible with state-of-the-art columns packed with sub-2µm particles (UHPSFC) has contributed to the reemergence of interest in this technology at the analytical scale. However, to ensure performance competitiveness of this technique with modern analytical standards, a robust hyphenation of UHPSFC to mass spectrometry (MS) is mandatory. UHPSFC-MS hyphenation interface should be able to manage the compressibility of the SFC mobile phase and to preserve as much as possible the chromatographic separation integrity. Although several interfaces can be envisioned, each will have noticeable effects on chromatographic fidelity, flexibility and user-friendliness. In the present study, various interface configurations were evaluated in terms of their impact on chromatographic efficiency and MS detection sensitivity. An interface including a splitter and a make-up solvent inlet was found to be the best compromise and exhibited good detection sensitivity while maintaining more than 75% of the chromatographic efficiency. This interface was also the most versatile in terms of applicable analytical conditions. In addition, an accurate model of the fluidics behavior of this interface was created for a better understanding of the influence of chromatographic settings on its mode of operation. In the second part, the most influential experimental factors affecting MS detection sensitivity were identified and optimized using a design-of-experiment approach. The application of low capillary voltage and high desolvation temperature and drying gas flow rate were required for optimal ESI ionization and nebulization processes. The detection sensitivity achieved using the maximized UHPSFC-ESI-MS/MS conditions for a mixture of basic pharmaceutical compounds showed 4- to 10-fold improvements in peak intensity compared to the best performance achieved by UHPLC-ESI-MS/MS with the same MS detector.

http://dx.doi.org/10.1016/j.chroma.2014.03.006

279. The Modeling Of Overloaded Elution Band Profiles In Supercritical Fluid Chromatography

Keywords: University of Tennessee, average volumetric flow rate, equilibrium dispersive model, separation science

Abstract
Three methods were used to analyze elution bands of methanol on silica, using pure CO2 as the eluent. The results of these analyses were applied to calculate overloaded elution band profiles in supercritical fluid chromatography. The results obtained are compared. To ensure that the mobile phase density varies widely along the column bed, high volumetric flow rates of the mobile phase (CO2) were applied to two columns packed with neat, porous silica. Then, even a slight error made in the determination of the isotherm parameters or during the numerical calculations should be magnified compared to those obtained with a low pressure drop along the column. During the determination of the isotherms of adsorption of methanol from liquid carbon dioxide onto silica, the inlet and outlet pressure of the column, the mass flow rate and the temperature were monitored continuously. Based on these parameters, overloaded elution bands were calculated numerically using three calculation methods. The results are compared with experimental ones. [http://dx.doi.org/10.1016/j.chroma.2014.01.034]

**280. Combined Size Exclusion Chromatography, Supercritical Fluid Chromatography And Electrospray Ionization Mass Spectrometry For The Analysis Of Complex Aliphatic Polyesters**

Keywords: University of Stellenbosch, aliphatic polyesters, chemical materials

**Abstract**

Aliphatic polyesters are complex products of polycondensation that are distributed regarding the degree of polycondensation, the end group functionality and the molecular topology. To address the molecular heterogeneity of polyesters based on phthalic acid and propylene glycol, for the first time the combination of SEC, SFC and ESI-MS have been used. In a first set of experiments, samples were fractionated by SEC and the collected fractions analyzed by SFC for a tentative assignment of the degrees of polycondensation. More conclusive results were obtained by semi-preparative SFC fractionation of the bulk samples and the subsequent analysis of the collected fractions by ESI-MS. The ESI-MS spectra of the SFC fractions provided detailed information on the presence of linear and cyclic oligomers, their degrees of polycondensation and their end groups. Information on the presence of propylene oxide oligomers was also obtained and it was shown how they were inserted in the polymer structures. Compared to previous work, the present approach provides significantly more detailed information on the molecular complexity of aliphatic polyesters. This is mainly due to the fact that SFC has been used as the second chromatographic dimension which is known to have superior separation capabilities. [http://dx.doi.org/10.1016/j.chroma.2014.01.018]

**281. Comparative Assessment Of Achiral Stationary Phases For High Throughput Analysis In Supercritical Fluid Chromatography**

Keywords: Bristol Myers Squibb, residual silanols, ammonium acetate, separation science

**Abstract**

Supercritical fluid chromatography (SFC) using open bed fraction collection is becoming more widely used for purification of diverse collections of compounds for drug discovery. This application requires predictable chromatography on scale up from analytical to preparative conditions. We have discovered that the selectivities of many columns used for SFC change over time when ammonium acetate additive is present in the mobile phase as a result of changes in silanophilic interactions. This makes scale up predictions difficult. To address this challenge we have developed a nontraditional comprehensive column ranking. Our system is based on the long-term retention repeatability of basic drugs in ammonium acetate containing mobile phase. The decreases in retention over time were used as a measure of changing silanophilicity of the stationary phases and became the basis for a column ranking system. This system, along with results for 24 commonly used silica-based columns, is presented in this paper. [http://dx.doi.org/10.1016/j.chroma.2014.01.060]

**282. Ultra High Resolution SFC - MS As A High Throughput Platform For Metabolic Phenotyping: Application To Metabolic Profiling Of Rat And Dog Bile**

Keywords: Waters, Imperial College, King’s College, metabolic profiling, metabonomics, metabolomics, bile acids, bile metabolites, health science
Abstract
Ultra high resolution SFC-MS (on sub-2μm particles) coupled to mass spectrometry has been evaluated for the metabolic profiling of rat and dog bile. The selectivity of the SFC separation differed from that seen in previous reversed-phase UPLC-MS studies on bile, with the order of elution for analytes such as e.g., the bile acids showing many differences. The chromatography system showed excellent stability, reproducibility and robustness with relative standard deviation of less than 1% for retention time obtained over the course of the analysis. SFC showed excellent chromatographic performance with chromatographic peak widths in the order of 3s at the base of the peak. The use of supercritical fluid carbon dioxide as a mobile phase solvent also reduced the overall consumption of organic solvent by a factor of 3 and also reduced the overall analysis time by a factor of 30% compared to reversed-phase gradient LC. SFC-MS appear complementary to RPLC for the metabolic profiling of complex samples such as bile.
http://dx.doi.org/10.1016/j.jchromb.2014.04.017

283. Profiling Of Regioisomeric Triacylglycerols In Edible Oils By Supercritical Fluid Chromatography/Tandem Mass Spectrometry

Abstract
In this study, supercritical fluid chromatography (SFC) coupled with triple quadrupole mass spectrometry was applied to the profiling of several regioisomeric triacylglycerols (TAGs). SFC conditions (column, flow rate, modifier) were optimized for the effective separation of TAGs. In the column test, a triacontyl (C30) silica gel reversed-phase column was selected to separate TAG regioisomers. Multiple reaction monitoring was used to selectively quantify each TAG. Then, the method was used to perform detailed characterization of a diverse array of TAGs in palm and canola oils. Seventy TAGs (C46:0-C60:2) of these oils were successfully analyzed as a result, and twenty isomeric TAG pairs were separated well. In particular, this method provided the fast and high resolution separation of six regioisomeric TAG pairs (PPLn/PLnP, PPL/PLP, PPO/POP, SPLn/SLnP, SPO/SOP, SSO/SOS-stearic acid (S, 18:0), oleic acid (O, 18:1), linoleic acid (L, 18:2), linolenic acid (Ln, 18:3), palmitic acid (P, 16:0)) in a short time (50min) as compared to high performance liquid chromatography. We were able to demonstrate the utility of this method for the analysis of regioisomeric TAGs in edible oils.
http://dx.doi.org/10.1016/j.jchromb.2014.01.040

284. Chiral Separation Of A Diketopiperazine Pheromone From Marine Diatoms Using Supercritical Fluid Chromatography

Abstract
The proline derived diketopiperazine has been identified in plants, insects and fungi with unknown function and was recently also reported as the first pheromone from a diatom. Nevertheless the stereochemistry and enantiomeric excess of this natural product remained inaccessible using direct analytical methods. Here we introduce a chiral separation of this metabolite using supercritical fluid chromatography/mass spectrometry. Several chromatographic methods for chiral analysis of the diketopiperazine from the diatom Seminavis robusta and synthetic enantiomers have been evaluated but neither gas chromatography nor high performance liquid chromatography on different chiral cyclodextrin phases were successful in separating the enantiomers. In contrast, supercritical fluid chromatography achieved baseline separation within four minutes of run time using amylase tris(3,5-dimethylphenylcarbamate) as stationary phase and 2-propanol/CO2 as mobile phase. This very rapid chromatographic method in combination with ESI mass spectrometry allowed the direct analysis of the cyclic dipeptide out of the complex sea water matrix after SPE enrichment. The method could be used to determine the enantiomeric excess of freshly released pheromone and to follow the rapid degradation observed in diatom cultures. Initially only trace amounts of c(d-Pro-d-Pro) were found besides the dominant c(l-Pro-l-
Pro) in the medium. However the enantiomeric excess decreased upon pheromone degradation within few hours indicating that a preferential conversion and thus inactivation of the l-proline derived natural product takes place.

http://dx.doi.org/10.1016/j.jchromb.2013.12.040

285. Development Of A Supercritical Fluid Chromatography-Tandem Mass Spectrometry Method For The Determination Of Lacidipine In Beagle Dog Plasma And Its Application To A Bioavailability Study

2014 - Journal of Chromatography B

Keywords: Shenyang Pharmaceutical University, lacidipine, plasma, health science

Abstract

A simple, novel, rapid and sensitive supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) method was developed and validated for the determination of lacidipine in beagle dog plasma with nimodipine as internal standard. The method involved a simple liquid-liquid extraction method with tert-butyl methyl ether. The analytes were analyzed on an Acquity UPLC(2) with a HSS C18 SB column (3mm×100mm, 1.8μm) set at 50°C. The mobile phase was carbon dioxide (≥99.99%) and methanol (92:8, v/v) at a flow rate of 2ml/min, the compensation solvent was methanol with 2% formic acid at a flow rate of 0.2ml/min and a total analysis time of 1.5min for each sample. The multiple reaction-monitoring mode was used for quantification of ion transitions at m/z 473.32 → 354.10 and 419.00 → 343.10 for lacidipine and internal standard, respectively. The linearity range of proposed method was 0.10-100ng/ml (r(2)≥0.9990). The intra- and inter-day precision values were less than 15% and accuracy was from -0.83% to 3.27% at all quality control levels. The proposed method was successfully applied to a pharmacokinetic study of lacidipine in beagle dogs.

http://dx.doi.org/10.1016/j.jchromb.2013.11.029

286. Development And Validation Of A Supercritical Fluid Chromatography Method For The Direct Determination Of Enantiomeric Purity Of Provitamin B5 In Cosmetic Formulations With Mass Spectrometric Detection

2014 - Journal of Pharmaceutical and Biomedical Analysis

Keywords: Orleans University, chiral separation, enantiomeric purity, dexpanthenol, cosmetics, chiralpak, adsorbex, chemical materials

Abstract

A rapid and efficient chiral supercritical fluid chromatography (SFC) method has been developed for the quantitative determination of panthenol enantiomers in cosmetic formulations (cream, lotion, wipe, and exfoliant). Indeed, the pharmacological effect only depends on the D form (Dexpanthenol) thus accurate measurement of its enantiomeric purity in formulated cosmetic products is of interest. The samples were prepared with liquid-liquid extraction followed by solid-phase extraction on Adsorbex amino cartridges. After testing several enantioselective columns in an attempt at reversing the elution order to have the minor enantiomer eluted first, the best separation of enantiomers and internal standard (N-acetyl-L-alanine) was achieved on a 3 μm -amylose-type immobilized polysaccharide chiral stationary phase (Chiralpak IA) in less than 6 min with a simple mobile phase comprising carbon dioxide and 11% methanol pumped at 2.3 mL/min, 25°C and 150 bar backpressure. Supercritical fluid chromatography coupled to both an optical diode-array detector and a user-friendly single-quadrupole mass spectrometer (Waters QDa) equipped with electrospray ionization source has been used. The on-line coupling ensures the technique to be more informative and improves detection sensitivity, as underivatized panthenol has a poor UV absorption. The limit of quantification (LOQ) achieved with single-ion recording was 0.5 μg/mL. The method was validated in terms of linearity, precision and accuracy and satisfactory results were obtained.

http://dx.doi.org/10.1016/j.jpba.2014.09.036


2014 - Journal of Pharmaceutical and Biomedical Analysis
Keywords: Shenyang Pharmaceutical University, bifendate, pharmacokinetics, beagle dog plasma, health science

**Abstract**

In order to evaluate the pharmacokinetic characteristics of a new formulation of a bifendate solid dispersion in beagle dogs, a novel, sensitive and rapid supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) method was established and validated. Plasma samples were subjected to liquid-liquid extraction with ethyl acetate. Separation of bifendate and diazepam (internal standard, IS) was performed on an HSS C18 SB column (3×100mm, 1.8μm) with a mobile phase consisting of CO2 (≥99.99%) - methanol (95:5, v/v) at a flow rate of 2mL/min and the compensation solvent was methanol with 2% formic acid at a flow rate of 0.2mL/min. A tandem triple quadrupole mass spectrometer was operated in multiple reaction monitoring (MRM) mode with an electrospray ionization (ESI) source. Quantification of the ion transitions was at m/z 419.2→387.0 and 284.5→193.2 for bifendate and IS, respectively. The method was sensitive with a lower limit of quantification (LLOQ) of 0.5ng/mL and linear over the concentration range 0.5-250ng/mL. All the validation data, such as precision, accuracy, extraction recovery and matrix effect, were all within acceptable criteria. The method has been successfully applied to a pharmacokinetic study of bifendate in beagle dogs after oral administration of a bifendate solid dispersion.

http://dx.doi.org/10.1016/j.jpba.2014.05.030

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**288. Robust Method Optimization Strategy—A Useful Tool For Method Transfer: The Case Of SFC**

Keywords: University of Liege, QBD, design space, method optimization, inter-laboratory method transfer, separation science

**Abstract**

The concept of Quality by Design (QbD) is now well established in pharmaceutical industry and should be applied to the development of any analytical methods. In this context, the key concept of Design Space (DS) was introduced in the field of analytical method optimization. In chromatographic words, the DS is the space of chromatographic conditions that will ensure the quality of peaks separation, thus DS is a zone of robustness. In the present study, the interest of robust method optimization strategy was investigated in the context of direct method transfer from sending to receiving laboratory. The benefit of this approach is to speed up the method life cycle by performing only one quantitative validation step in the final environment of method use. A Supercritical Fluid Chromatography (SFC) method previously developed was used as a case study in this work. Moreover, the interest of geometric transfer was investigated simultaneously in order to stress a little bit more the transfer exercise and, by the way, emphasize the additional benefit of DS strategy in this particular context. Three successful transfers were performed on two column geometries. In order to compare original and transferred methods, the observed relative retention times (RT) were modelled as a function of the predicted relative RT and of the method type (original or transferred). The observed relative RT of the original and transferred methods are not statistically different and thus the method transfer is successfully achieved thanks to the robust optimization strategy. Furthermore, the analytical method was improved considering analysis time (reduced five times) and peak capacity (increased three times). To conclude, the
advantage of using a DS strategy implemented for the optimization and transfer of SFC method was successfully demonstrated in this work. http://dx.doi.org/10.1016/j.jpba.2013.09.030

289. Determination Of Coumarins In The Roots Of Angelica Dahurica By Supercritical Fluid Chromatography

Keywords: University of Innsbruck, ABPR, automated backpressure regulator, DGC, dense gas chromatography, HPGC, high pressure gas chromatography, SFC, supercritical fluid chromatography, SFE, supercritical fluid extraction, TCM, traditional Chinese medicine, TRPV1, transient receptor potential vanilloid type 1

Abstract
The fact that supercritical fluid chromatography (SFC) offers many desirable features is known for a long time. Yet, the number of applications on natural products is still limited, because robust and user-friendly instrumentation became available just a few years ago. As coumarins hardly have been studied by this technique we developed the first SFC assay for their determination in crude plant material. After method optimization eight standard compounds, including simple coumarins, linear and angular furanocoumarins, could be baseline separated in 6 min using an Acquity UPC2 CSH Fluoro-Phenyl 1.7 μm column with supercritical CO2, methanol and diethylamine as mobile phase. Method validation confirmed that the assay is linear (R² ≥ 0.9999), precise (intra-day variation ≤ 5.8%; inter-day variation ≤ 4.4%) and accurate (recovery rates from 96.5 to 104.2%). Detection limits determined at 300 nm were below 2 ng on-column, and the method showed to be well suited for the analysis of coumarins in Angelica dahurica roots. It was observed that qualitative as well as quantitative composition vary significantly. In all samples Imperatorin (0.09–0.28%) was the major coumarin, followed either by Isoimperatorin or Oxypeucedanin; the total coumarin content ranged from 0.16 to 0.77%. The results were in good agreement to published data, so that because of its speed and green nature SFC is definitely an interesting alternative for the analysis of this important class of natural products. http://dx.doi.org/10.1016/j.jpba.2016.07.014

290. Chromatographic Resolution Of Closely Related Species: Drug Metabolites And Analogs

Keywords: Merck, method development, high throughput analysis, drugs, metabolites, arbamazepine, methylated xanthine, steroid hormone, nicotine, morphine, GreenSep Ethyl, Luna HILIC, ChiralPak, pharmaceutical

http://dx.doi.org/10.1016/j.seppur.2014.03.018
Abstract
In this study, we investigate the separation of a variety of mixtures of drugs, metabolites, and related analogs including representatives of the carbamazepine, methylated xanthine, steroid hormone, nicotine, and morphine families using several automated chromatographic method development screening systems including ultra high performance liquid chromatography, core–shell HPLC, achiral supercritical fluid chromatography (SFC), and chiral SFC. Of the 138 column and mobile phase combinations examined for each mixture, a few chromatographic conditions afford the best overall performance, with a single achiral SFC method (4.6 × 250 mm, 3.0 μm GreenSep Ethyl Pyridine, 25 mM isobutylamine in methanol/CO₂) affording good separation for all samples. Four of these mixtures were also resolved by achiral SFC on the Luna HILIC and chiral SFC Chiralpak IB columns using methanol or ethanol with 25 mM isobutylamine as polar modifiers. Modifications of standard chromatography screening conditions afforded fast separation methods (from 1 to 5 min) for baseline resolution of all components of each of these challenging sets of closely related compounds.
http://dx.doi.org/10.1002/jssc.201400038

291. Development Of An Automated Dual-Mode Supercritical Fluid Chromatography And Reversed-Phase Liquid Chromatography Mass - Directed Purification System For Small-Molecule Drug Discovery

Keywords: Theravance, chiral separation, achiral separation, pharmaceutical

Abstract
We report the development of a dual-mode mass-directed supercritical fluid chromatography and reversed-phase liquid chromatography purification system. The addition of a third pump allows for flexible mobile phase control between the two techniques, and enables operation of either chromatography mode within minutes by activation of a set of switching valves on a single system. Software control, fluidic pathways, interface to the mass spectrometer, and fraction collection have been modified for compatibility between both separation methods. The conditioning solvent and tuning parameters for the mass spectrometer were adjusted to achieve an ideal signal trace in either mode with good linearity ($r^2 > 0.970$) over a range of concentrations and minimal noise for accurate peak detection and isolation. The registration success rate is 90% and overall sample recovery for either technique is 80–90%. Combining two orthogonal separation and purification modes in one single system has improved the purification throughput of complex mixtures and has been a valuable, cost-saving tool in our laboratory.
http://dx.doi.org/10.1002/jssc.201301366

292. Supercritical Fluid Extraction And Convergence Chromatographic Determination Of Parthenolide In Tanacetum Parthenium L.: Experimental Design, Modeling And Optimization

Keywords: Semmelweis University, medicinal plant, sesquiterpene-γ-lactones, flavonoids, volatile oil, parthenolide, natural products, health science

Abstract
Feverfew (Tanacetum parthenium L., Asteraceae) is a perennial medicinal plant which has been used to alleviate the symptoms of migraines, headaches and rheumatoid arthritis. The herb contains various potentially active constituents such as sesquiterpene-γ-lactones, flavonoids and volatile oil. The main sesquiterpene-lactone in feverfew is parthenolide which is considered to be responsible for the therapeutical effects. Supercritical CO₂ extraction was carried out at different pressures (10–30 MPa), temperatures (40–80 °C) and co-solvent contents (0–10% ethanol) in order to study the extraction yield and the parthenolide recovery of the extracts. Leaves collected before and during flowering and flower heads were investigated. A factorial experiment using a full 3² design was followed during the experiments and response surface methodology was implemented to analyze the influence of the variables and optimize the extraction. The critical values of parthenolide content were found to be 7% EtOH, 22 MPa and 64 °C in case of all three samples. It was determined, that the optimal conditions of the extraction, where the maximum parthenolide content and extract yield can be reached, do not coincide. The highest yield of parthenolide was obtained in the flower heads (0.604 wt.%).
http://dx.doi.org/10.1016/j.supflu.2014.07.029
293. Determination Of Niacin And Its Metabolites Using Supercritical Fluid Chromatography Coupled To Tandem Mass Spectrometry


Keywords: Osaka University, water soluble vitamin, vitamin B, hydrophilic metabolites, biofluid, pharmaceutical

Abstract
Niacin, a water-soluble vitamin belonging to the vitamin B group, has been known to cause various problems in the human body when deficient. The vitamin is derived from the diet and afterwards, niacin and its metabolites are secreted in blood or urine. It can be analyzed using liquid chromatography (LC) coupled to mass spectrometry, but niacin and its metabolites are very polar compounds. Recently, supercritical fluid chromatography (SFC) is gaining attention for polar compound analysis. To our best knowledge, the report on the analysis of endogenous-very hydrophilic metabolites in biofluids by SFC has not been found. In this study, we investigated whether the separation of hydrophilic metabolites in biofluids is achievable by SFC. In addition, we also examined the applicability of SFC coupled to MS in extrapolating unknown metabolites by means of spectra information. As a result, an analysis method to quantify the target compounds using SFC/MS/MS was constructed for niacin and its metabolites. Additional putative metabolites from niacin were also identified using the MS fragmentation spectra in plasma and urine. Consequently, the method using SFC-MS/MS can be an alternative technique for hydrophilic metabolite analysis.

http://dx.doi.org/10.5702/massspectrometry.A0029

294. Review Article: Support Of Academic Synthetic Chemistry Using Separation Technologies From The Pharmaceutical Industry

2014 - Organic and Biomolecular Chemistry

Keywords: Merck, University of Pennsylvania, Harvard University, University of Missouri, Scripps Research Institute, achiral screening, chiral screening, chiral loading and purification, pharmaceutical

Abstract
The use of state-of-the-art separation tools from the pharmaceutical industry for addressing intractable separation problems from academic synthetic chemistry is evaluated, showing fast and useful results for the resolution of complex mixtures, separation of closely related components, visualization of difficult to detect compounds and purification of synthetic intermediates. Some recommendations for potential near term deployment of separation tools within academia and the evolution of next generation separation technologies are discussed.

http://dx.doi.org/10.1039/C3OB42195C

Keywords: Charles University, Steroids, estrogens, health science

Abstract

Estrogen steroids, represented by estradiol and its related substances, include both structurally very close and simultaneously different analogs. Their separation still remains an analytical challenge. Subcritical fluid chromatography (SbFC) on sub-2-micron particles was found to be an appropriate tool to obtain fast and efficient separation of nine target analytes. Among the four tested stationary phases charged hybrid modified with PFP (pentafluorophenyl) moiety was found to be the most convenient providing the fastest separation within 1.6 min using quick gradient elution with carbon dioxide and methanol as an organic modifier. However, complete separation was obtained also on other tested phases including bare hybrid stationary phase, hybrid stationary phase modified with 2-EP (2-ethylpyridine) and also C18, which is less typical in SbFC. The baseline separation on the latter columns was achieved by means of a temperature increase, a change in organic modifier type and gradient time increase respectively.

Quantitative performance was evaluated at optimized conditions and method validation was accomplished. Excellent repeatability of both retention times (RSD<0.15%) and peak areas (RSD<1%) was observed. The method was linear in the range of 1.0–1000.0 μg/ml for all steroids with the lowest calibration point being an LOQ, except for Δ-derivatives, that provided better sensitivity and thus LOQ of 0.5 μg/ml. The sensitivity was sufficient for the analysis of real samples although it was still five times lower compared to UHPLC-UV experiments.

http://dx.doi.org/10.1016/j.talanta.2013.12.056

296. Simultaneous Determination Of 17 Disperse Dyes In Textile By Ultra-High Performance Supercritical Fluid Chromatography Combined With Tandem Mass Spectrometry

2014 - Talanta
Keywords: Beijing University of Chemical Technology, dyes, textiles, BEH, BEH 2-ethyl-pyridine, HSS C18 SB and CSH fluorophenyl, chemical materials

Abstract
A simple, highly sensitive and fast procedure for the control of 17 allergenic and prohibited disperse dyes in textile products was optimized. The method was based on ultrasound assisted extraction of textile samples with 10 mL of methanol under controlled conditions (30 min, 70°C). The extracts were analyzed by the ultra-high performance supercritical fluid chromatography (UHPSFC) system coupled with triple quadrupole tandem mass spectrometry (MS/MS). Four stationary phases (BEH, BEH 2-ethyl-pyridine, HSS C18 SB and CSH fluorophenyl) were screened as well as analytical conditions (modifier percentage, backpressure and column temperature) were investigated to improve the separation. All 17 disperse dyes were simultaneously separated and determined by UHPSFC-MS/MS in 5 min. The dyes were monitored via the ESI(+) ionization method and quantified by 3-channel multiple reaction monitoring (MRM). The calibrations were performed and good linear relationship (R≥0.99) was observed within the concentration range of 2-50 μg mL(-1). Satisfactory recoveries (70.55-103.03%) of all the disperse dyes spiked with standards at different levels were demonstrated. This is the first report on the simultaneous analysis of disperse dyes using UHPSFC-MS/MS.

http://dx.doi.org/10.1016/j.talanta.2014.03.055

297. Synthesis Of Dopamine D2/D3 Receptor Agonist (+)-PHNO Via Super Critical Fluid Chromatography: Preliminary PET Imaging Study With [3-11C]-(+) PHNO

Keywords: Massachusetts General Hospital, Harvard Medical School, Waters, PHNO, Carbon-11, dopamine D3 agonist, [11C]methyl iodide, brown fat, health science,

Abstract
Carbon-11 labeled (+)-4-[1-11C]propyl-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-b][1,4]oxazin-9-ol (([1-11C]-(+)-PHNO) is a dopamine D3-preferring agonist radiopharmaceutical used for medical imaging by positron emission tomography (PET). We report the synthesis of (+)-PHNO using supercritical fluid chromatography for enantiomeric resolution of its norpropyl derivative, HNO, followed by propylation. (+)-HNO was used to prepare the radiolabeling precursor, (+)-trans-4-acetyl-9-trisopropylsilyloxy-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine, in 12 steps. Modifications to the labeling procedure were made to ensure consistent preparation of [3-11C]-(+)-PHNO via [11C]CH3I. A preliminary PET imaging study was carried out with this tracer in an attempt to image dopamine receptors in brown adipose tissue (brown fat) in vivo.

http://dx.doi.org/10.1016/j.tetlet.2013.11.113

298. The Use Of Columns Packed With Sub-2-µm Particles In Supercritical Fluid Chromatography

Keywords: Waters, supercritical fluid chromatography, Waters, sub-2-µm columns, chemical materials
Abstract
The development of greener analytical techniques is a topic of great interest and in this context, supercritical fluid chromatography (SFC) is making an interesting comeback. The present review focuses on the latest developments of this technique. Improvements brought to the chromatographic systems and requirements to achieve full compatibility with columns packed with sub-2 μm particles (UHPSFC) are discussed. A thorough kinetic evaluation made using van Deemter representations, isopycnic and kinetic plots shows that performance achieved with state-of-the-art UHPSFC hardware and columns is comparable to that obtained in UHPLC. Orthogonal selectivity compared to reversed phase LC (RPLC) and extended selectivity modulation possibilities achievable in supercritical conditions using different stationary phase chemistries are presented. Finally, current applications of UHPSFC and its different hyphenation possibilities including mass spectrometry (MS) are also reviewed, in the hope of helping chromatography users to have a new look on the possibilities offered by this technique.
http://dx.doi.org/10.1016/j.trac.2014.06.023
299. Chromatographic Resolution Of Closely Related Species In Pharmaceutical Chemistry: Dehalogenation Impurities And Mixtures Of Halogen Isomers

Keywords: Merck, halogen isomers, dehalogenated isosteres, Hypersil Gold PFP, Chiralcel, Chiralpak, Zorbax, pharmaceutical

Abstract

In recent years, the use of halogen-containing molecules has proliferated in the pharmaceutical industry, where the incorporation of halogens, especially fluorine, has become vitally important for blocking metabolism and enhancing the biological activity of pharmaceuticals. The chromatographic separation of halogen-containing pharmaceuticals from associated isomers or dehalogenation impurities can sometimes be quite difficult. In an attempt to identify the best current tools available for addressing this important problem, a survey of the suitability of four chromatographic method development platforms (ultra high-performance liquid chromatography (UHPLC), core shell HPLC, achiral supercritical fluid chromatography (SFC) and chiral SFC) for separating closely related mixtures of halogen-containing pharmaceuticals and their dehalogenated isosteres is described. Of the 132 column and mobile phase combinations examined for each mixture, a small subset of conditions were found to afford the best overall performance, with a single UHPLC method (2.1 × 50 mm, 1.9 μm Hypersil Gold PFP, acetonitrile/methanol based aqueous eluents containing either phosphoric or perchloric acid with 150 mM sodium perchlorate) affording excellent separation for all samples. Similarly, a survey of several families of closely related halogen-containing small molecules representing the diversity of impurities that can sometimes be found in purchased starting materials for synthesis revealed chiral SFC (Chiralcel OJ-3 and Chiralpak IB, isopropanol or ethanol with 25 mM isobutylamine/carbon dioxide) as well as the UHPLC (2.1 × 50 mm, 1.8 μm ZORBAX RRHD Eclipse Plus C18 and the Gold PFP, acetonitrile/methanol based aqueous eluents containing phosphoric acid) as preferred methods.

http://dx.doi.org/10.1021/ac403376h

300. Development Of Supercritical Fluid (Carbon Dioxide) Based Ultra Performance Convergence Chromatographic Stability Indicating Assay Method For The Determination Of Clofarabine In Injection

Keywords: Mylan Labs, clofarabine, stability indicating method, BEH 2-EP, pharmaceutical

Abstract

The present study reports the development and validation of a stability indicating assay method for clofarabine in injection on a UPC²™ (ultra performance convergence chromatography) instrument, which utilizes the unrealized potential of supercritical fluid chromatography. The use of UPC²™ provides a single viable technique that is a sustainable, reduced cost, and green technology that lowers the use of organic solvents. Based on this advantage, we explored a simple and robust method in order to increase sample throughput and productivity to quantify clofarabine in the presence of its potential impurities and other degradants. The separation was achieved on a BEH-2-ethyl pyridine (BEH 2EP) column (100 mm × 3.0 mm I.D. with an average pore diameter of 1.7 μm) by using methanol as a co-solvent and carbon dioxide as a mobile phase in the ratio of 30 : 70. The detection is carried out at a wavelength of 254 nm. We are able to achieve the separation of clofarabine from its potential impurities and other degradants in less than 6 minutes with a low amount of solvent consumption. The new method is validated in accordance with the ICH-guidelines and exhibited good intra- and inter-day precision, accuracy and linearity (r² ≥ 0.999) over a range of 50% to 150% of target concentration.

http://dx.doi.org/10.1039/C3AY41561A
301. Supercritical Fluid (Carbon Dioxide) Based Ultra Performance Convergence Chromatography For The Separation And Determination Of Fulvestrant Diastereomers

Keywords: Mylan Labs, fluvestrant diastereomers, stability indicating method, BEH 2-EP, pharmaceutical

Abstract
UltraPerformance convergence chromatography (UPC²™) is a new category of separation science which utilizes the unrealized potential of the supercritical chromatography phenomenon. UPC²™ is a stand-alone, viable technique that is cost effective, sustainable, and uses green technology that lowers the use of organic solvents. Based on this advantage, we explored a simple and robust supercritical liquid-based UPC² method in order to increase sample throughput and productivity to quantify the diastereomers of fulvestrant. The two isomers of fulvestrant were well separated on a chiral column (150 mm × 4.6 mm, I.D.) by applying a mixture of methanol and acetonitrile (9.5 : 0.5) as the co-solvent of the mobile phase of carbon dioxide (75%). The detection was carried out at 280 nm. We were able to achieve a three-fold reduction in retention with an isocratic mode as compared to the United States Pharmacopeias (USP) normal phase method. This new method was validated in accordance with the ICH guidelines; it exhibited good intra- and inter-day accuracy, precision, and the results were linear over a range of 25% to 150% of the target concentration. The method could be successfully applied for the determination of the diastereomeric ratio of fulvestrant as an API and in fulvestrant injectable finished products.
http://dx.doi.org/10.1039/C3AY40802G

302. Organocatalytic Enantioselective Cycloaddition Reactions Of Dienamines With Quinones

Keywords: Aarhus University, organocatalysis, quinones, separation science

Abstract
Matching catalyst and substrate: Organocatalytic cycloaddition between dienamines and 1,4-benzo- or 1,4-naphthoquinones affords biologically interesting dihydroanaphtho- and dihydroanthraquinone core structures. The enantioselectivity of this new reaction is ensured by a steric shielding catalyst and carefully selecting substrates that greatly favor the endo approach (see scheme) due to electrostatic interactions in the zwitterionic intermediate.
303. Breaking Symmetry With Symmetry: Bifacial Selectivity In The Asymmetric Cycloaddition Of Anthracene Derivatives

Keywords: Aarhus University, anthracene, separation science

Abstract
A new catalytic strategy for the activation of anthracene derivatives has been developed. From symmetrical starting materials, enantioselective cycloaddition reactions can be achieved by employing a $C_2$-symmetric aminocatalyst. This selectivity is due to the gain or loss of conjugation between the enamine and the anthracene in the two transition-state structures. This methodology is demonstrated in 14 examples with 70–96% yield and 76–95% ee.

http://dx.doi.org/10.1002/chem.201300142

304. Chromatographic Separation And Assignment Of Absolute Configuration Of Hydroxywarfarin Isomers

Keywords: Merck, hydroxywarfarin isomers, chiral SFC, human liver microsomes, pharmaceuticals

Abstract
The absolute configuration of several hydroxywarfarin isomers was assigned using a comparison of elution order on chiral stationary phases, optical rotation, and circular dichroism (CD) spectra, with confirmation of assignments made by comparison between experimental and calculated CD spectra and selective synthesis of hydroxywarfarin isomers from enantiopure warfarin using human liver microsomes.

http://dx.doi.org/10.1002/chir.22274

305. Improved Chiral SFC Screening For Analytical Method Development

Keywords: Merck, chiral sfc, method development, Chiralpak, Chiralcel, Phenomenex, pharmaceutical

Abstract
In this study we describe the evaluation of a recently developed supercritical fluid chromatography (SFC) instrument for automated chiral SFC method development. The greatly improved gradient dwell volume and liquid flow control of the new instrument in combination with the use of shorter columns containing smaller stationary phase particles affords chiral SFC method development that is faster and more universal than previous systems.

http://dx.doi.org/10.1002/chir.22218

306. Application Of 'Omic Technologies To Biomarker Discovery In Inflammatory Lung Diseases

Keywords: Karolinska Institute, omics, biomarker discovery, lung disease, health science

Abstract
Inflammatory lung diseases are highly complex in respect of pathogenesis and relationships between inflammation, clinical disease and response to treatment. Sophisticated large-scale analytical methods to quantify gene expression (transcriptomics), proteins (proteomics), lipids (lipidomics) and metabolites (metabolomics) in the lungs, blood and urine are now available to identify biomarkers that define disease in terms of combined clinical, physiological and patho-
biological abnormalities. The aspiration is that these approaches will improve diagnosis, i.e. define pathological phenotypes, and facilitate the monitoring of disease and therapy, and also, unravel underlying molecular pathways. Biomarker studies can either select predefined biomarker(s) measured by specific methods or apply an “unbiased” approach involving detection platforms that are indiscriminate in focus. This article reviews the technologies presently available to study biomarkers of lung disease within the ‘omics field. The contributions of the individual ‘omics analytical platforms to the field of respiratory diseases are summarised, with the goal of providing background on their respective abilities to contribute to systems medicine-based studies of lung disease.

http://dx.doi.org/10.1183/09031936.00078812

307. Asymmetric Organocatalytic Thio-Diels–Alder Reactions Via Trienamine Catalysis

Keywords: Aarhus University, enantioselective synthesis, diasteroselective synthesis, separation science

Abstract

We report a new process to access highly enantioenriched sulfur-based heterocycles by an asymmetric catalytic thio-Diels–Alder reaction. Thiocarbonyls are challenging heterodienophiles in enantioselective Diels–Alder reactions, due to their inherent high reactivity and their poor ability to coordinate to chiral catalysts. We successfully circumvented these problems by employing a different strategy, which explores the use of in situ generated catalyst-bound dienes. Synthetically useful dihydrothiopyrans as well as other bi- and tricyclic sulfur-containing heterocycles are formed in high yields and high to excellent selectivities. DFT calculations were performed to examine the mechanism of the developed reaction. Furthermore, a series of synthetic transformations of the optically active sulfur-based heterocycles are presented.

http://dx.doi.org/10.1021/ja4007244

308. Characterization Of Five Chemistries And Three Particle Sizes Of Stationary Phases Used In Supercritical Fluid Chromatography

Keywords: University of Orleans, linear solvation energy parameters, BEH, XSelect, HSS, separation science

Abstract

Sub-2-microns particles employed as supporting phases are known to favor column efficiency. Recently a set of columns based on sub-2-microns particles for use with supercritical fluid mobile phases have been introduced by Waters. Five different stationary phase chemistries are available: BEH silica, BEHEthyl-pyridine, X Select CSH Fluorophenyl, HSS C18 SB and BEH Shield RP18. This paper describes the characterization of 15 stationary phases, the five different chemistries, and three particle sizes, 1.7 (or 1.8), 3.5 and 5 microns, with the same carbon dioxide–methanol mobile phase and a set of more than a hundred compounds. The interactions established in the 15 different chromatographic systems used in supercritical fluid chromatography (SFC) are assessed with linear solvation energy relationships (LSERs). The results show the good complementarity of the five column chemistries, and their comparative location inside a classification map containing
Waters

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today around 70 different commercial phases. Among the five different chemistries, the HSS C18 SB phase displays a rather unusual behavior in regards of classical C18 phases, as it displays significant hydrogen–bonding interactions. Besides, it appears, as expected, that the BEH Ethyl–pyridine phase has weak interactions with basic compounds. The effect of particle size was studied because smaller particles induce increased inlet and internal pressure. For compressible fluids, this pressure change modifies the fluid density, i.e. the apparent void volume and the eluting strength. These changes could modify the retention and the selectivity of compounds in the case of method trans-fer, by using different particle sizes, from 5 down to 1.7 m. A hierarchical cluster analysis shows that stationary phase clusters were based on the phase chemistry rather than on the particle size, meaning that method transfer from 5 to 1.7 microns can be achieved in the subcritical domain i.e. by using a weakly compressible fluid.

http://dx.doi.org/10.1016/j.chroma.2013.10.037

309. Accurate On-Line Mass Flow Measurements In Supercritical Fluid Chromatography

Keywords: University of Tennesse, Waters, separation science

Abstract

This work demonstrates the possible advantages and the challenges of accurate on-line measurements of the CO2 mass flow rate during supercritical fluid chromatography (SFC) operations. Only the mass flow rate is constant along the column in SFC. The volume flow rate is not. The critical importance of accurate measurements of mass flow rates for the achievement of reproducible data and the serious difficulties encountered in supercritical fluid chromatography for its assessment were discussed earlier based on the physical properties of carbon dioxide. In this report, we experimentally demonstrate the problems encountered when performing mass flow rate measurements and the gain that can possibly be achieved by acquiring reproducible data using a Coriolis flow meter. The results obtained show how the use of a highly accurate mass flow meter permits, besides the determination of accurate values of the mass flow rate, a systematic, constant diagnosis of the correct operation of the instrument and the monitoring of the condition of the carbon dioxide pump.

http://dx.doi.org/10.1016/j.chroma.2013.10.041

310. Maximizing Kinetic Performance In Supercritical Fluid Chromatography Using State-Of-The-Art Instruments

Keywords: University of Geneva, Genentech, extra-column band broadening, kinetic performance, separation science

Abstract

Recently, there has been a renewed interest in supercritical fluid chromatography (SFC), due to the introduction of state-of-the-art instruments and dedicated columns packed with small particles. However, the achievable kinetic performance and practical possibilities of such modern SFC instruments and columns has not been described in details until now. The goal of the present contribution was to provide some information about the optimal column dimensions (i.e. length, diameter and particle size) suitable for such state-of-the-art systems, with respect to extra-column band broadening and system upper pressure limit. In addition, the reliability of the kinetic plot methodology, successfully applied in RPLC, was also evaluated under SFC conditions. Taking into account the system variance, measured at ~85μL(2), on modern SFC instruments, a column of 3mm I.D. was ideally suited for the current technology, as the loss in efficiency remained reasonable (i.e. less than 10% decrease for k>6). Conversely, these systems struggle with 2.1mm I.D. columns (55% loss in N for k=5). Regarding particle size, columns packed with 5μm particles provided unexpectedly high minimum reduced plate height values (hmin=3.0-3.4), while the 3.5 and 1.7μm packing provided lower reduced plate heights hmin=2.2-2.4 and hmin=2.7-3.2, respectively. Considering the system upper pressure limit, it appears that columns packed with 1.7μm particles give the lowest analysis time for efficiencies up to 40,000-60,000 plates, if the mobile phase composition is in the range of 2-19% MeOH. The 3.5μm particles were attractive for higher efficiencies, particularly when the modifier percentage was above 20%, while 5μm was never kinetically relevant with modern SFC instruments, due to an obvious limitation in terms of upper flow rate value. The present work also confirms that the kinetic plot methodology could be...
successfully applied to SFC, without the need for isopycnic measurements, as the difference in plate count between predicted and experimental values obtained by coupling several columns in series (up to 400mm) was on average equal to 3-6% and with a maximum of 13%.

http://dx.doi.org/10.1016/j.chroma.2013.09.039

311. Supercritical Fluid Chromatography In Food Analysis
2013 - Journal of Chromatography A

Keywords: University of Valladolid, lipid, carotenoids, fat soluble vitamins, food

Abstract
In the last years, supercritical fluid chromatography (SFC) has increased its acceptance between scientists. The unique selectivity, short analysis times, low consumption of organic solvents as well as the improvements in instrumentation have contributed to expand its use. These characteristics make SFC a powerful tool when food analysis requires individualized evaluation of several compounds in very complex samples. In this work, the advantages and main applications of SFC in food analysis are reviewed, focusing special attention onto analytical and preparative separations.

http://dx.doi.org/10.1016/j.chroma.2013.07.022

312. Determination Of Adsorption Isotherms In Supercritical Fluid Chromatography
2013 - Journal of Chromatography A

Keywords: Karlstad University, adsorption isotherms, perturbation peak method, retention time method, inverse method, separation science

Abstract
In this study we will demonstrate the potential of modern integrated commercial analytical SFC-systems for rapid and reliable acquisition of thermodynamic data. This will be done by transferring the following adsorption isotherm determination methods from liquid chromatography (LC) to supercritical fluid chromatography (SFC): Elution by Characteristic Points (ECP), the Retention Time Method (RTM), the Inverse Method (IM) and the Perturbation Peak (PP) method. In order to transfer these methods to SFC in a reliable, reproducible way we will demonstrate that careful system verification using external sensors of mass flow, temperature and pressure are needed first. The adsorption isotherm data generated by the different methods were analyzed and compared and the adsorption isotherms ability to predict new experimental elution profiles was verified by comparing experiments with simulations. It was found that adsorption isotherm data determined based on elution profiles, i.e., ECP, IM and RTM, were able to accurately predict overloaded experimental elution profiles while the more tedious and time-consuming PP method, based on small injections on concentration plateaus, failed in doing so.

http://dx.doi.org/10.1016/j.chroma.2013.09.007

313. Strong Cation Exchange Chiral Stationary Phase – A Comparative Study In High-Performance Liquid Chromatography And Subcritical Fluid Chromatography
2013 - Journal of Chromatography A

Keywords: University of Vienna, Waters, Institute of Chemical Technology, enantiomer separation, chiral cation exchange, transition buffer salts, separation science

Abstract
The performance of a strong cation exchange-type (SCX) chiral stationary phase (CSP) was evaluated with subcritical fluid chromatography (subFC) and high performance liquid chromatography (HPLC). The chromatographic conditions in subFC were optimized by changing the amount of polar organic modifier, concentration of a basic additive in the modifier, system pressure and temperature. In this way the concentration of in situ formed transient ionic species could be varied. The gradual change of the concentration of the transient buffer, i.e. gradient elution conditions in subFC, was found beneficial for separation of a mixture of racemic compounds. The strength and amount of the in situ formed buffer was estimated on the basis of comparative experiments in subSFC and HPLC.

http://dx.doi.org/10.1016/j.chroma.2013.08.037
314. In-Depth Characterization Of Six Cellulose Tris-(3,5-Dimethylphenylcarbamate) Chiral Stationary Phases In Supercritical Fluid Chromatography

Keywords: University of Orleans, Bristol Myers Squibb, chemometrics, chiral recognition, quantitative structure retention relationships, solvation parameter model, separation science

Abstract
Since the expiration of the patent protection of Chiralcel OD, similar chiral stationary phases (CSPs), all based on the same chiral selector, have been introduced on the market with the promise to reproduce or improve the performance of the original cellulose tris-(3,5-dimethylphenylcarbamate) CSP. We report here-in an in-depth evaluation of four generic versions of Chiralcel OD (CelluCoat, RegisCell, Lux Cellulose-1, Reprosil-OM) and the immobilized version (Chiralpak IB) in comparison to the original Chiralcel OD in terms of retention and enantioselectivity, with the help of chemometrics. First of all, the CSPs are compared based on the retentions of 230 achiral compounds. Agglomerative hierarchical clustering and quantitative structure–retention relationships based on a modified version of the solvation parameter model are used to assess the differences in non-enantioselective interactions contributing to retention. Secondly, the CSPs are compared based on the separation factors measured for 130 racemates. Discriminant analysis is then used to unravel the structural features contributing to the successful enantioselective separations. Chiralcel OD is shown to be the most versatile of the six tested CSPs, and involves a unique and unequalled mechanism to achieve enantioseparation.

http://dx.doi.org/10.1016/j.chroma.2013.06.040

315. Chromatographic Resolution Of Closely Related Species: Separation Of Warfarin And Hydroxylated Isomers

Keywords: Merck, chiral screening, method development, pharmaceutical

Abstract
Recent developments in the field of organic synthesis are leading to increasingly complex mixtures of closely related species (positional isomers, regioisomers, diastereomers, etc.) that often prove challenging for chromatographic analysis and separation. In this study we investigate the separation of a representative mixture of warfarin and 5 different monohydroxylation isomers to assess whether conventional techniques are suitable for addressing this separation challenge, or whether 'next generation' separation tools such as multidimensional chromatography may be required. In this example, modifications of results obtained from conventional achiral and chiral chromatography method development screening platforms afford rapid separation of all components for both achiral and chiral analysis, with supercritical fluid chromatography showing the best performance in both cases (1.8min for separation of six components by achiral SFC and 8.0min for separation of twelve components by chiral SFC). While other more complex mixtures may require additional tools, these results suggest that new applications of existing separation platforms may be useful for creating the chromatographic methods required to support this new area of synthetic chemistry.

http://dx.doi.org/10.1016/j.chroma.2013.07.092

316. The Evaluation Of 25 Chiral Stationary Phases And The Utilization Of Sub-2.0 Mm Coated Polysaccharide Chiral Stationary Phases Via Supercritical Fluid Chromatography

Keywords: Genentech, chiral screening, polysaccharide chiral stationary phases, separation science

Abstract
A rapid screening method to identify the best conditions for chiral separations is described. We analyzed a representative set of 80 racemic compounds against 25 different chiral stationary phases with three different mobile phases to identify the combination of columns and mobile phases that will separate the most compounds on the initial screen. While the OD separated the largest number of compounds, we found the best combination of six columns to be the AD, AS, AY, CC4, ID and Whelk-O1. The second team included the CCC, Cellulose-1, Cellulose-3 or OJ, IA,
IE and IF. All 80 compounds were separated with a resolution range of 0.65–15.36. Screening the covalently bonded phases provided separation for 79 of the 80 compounds. We also found ethanol (0.1% NH₄OH) separated more compounds than methanol (0.1% NH₄OH) or isopropanol (0.1% NH₄OH). As part of this study, we also compared the effectiveness of stationary phases that have the same chiral selector. Finally, we demonstrated the effectiveness of using a fast, 1.5-min screening method that utilizes a 1.7 μm coated polysaccharide chiral stationary phase.

http://dx.doi.org/10.1016/j.chroma.2013.07.046

317. Simultaneous And Rapid Analysis Of Bile Acids Including Conjugates By Supercritical Fluid Chromatography Coupled To Tandem Mass Spectrometry

Keywords: Waters, Osaka University, metabolic profiling, health science

Abstract
A number of analysis methods for bile acids using LC-MS and GC-MS have been reported. However, there is no reported method for the simultaneous analysis of bile acids using supercritical fluid chromatography (SFC). In this study, we have successfully developed a rapid method using SFC coupled to electrospray ionization tandem mass spectrometry (ESI-MS/MS) for comprehensive bile acid profiling. 25 bile acids including glycine and taurine conjugates were analyzed simultaneously within 13 min. The method was applied to rat serum samples, 24 of the bile acids were quantified without any solid-phase extraction and complicated sample preparation. This study not only reports simultaneous analysis of bile acids including conjugates but also indicates the method is applicable to a biological sample. This is the first report on the simultaneous analysis of bile acids using SFC/MS. The developed method will be an alternative to existing analysis methodology for studies on the bile acid metabolism in the medical and pharmaceutical fields.

http://dx.doi.org/10.1016/j.chroma.2013.05.043

318. Strong Cation Exchange-Type Chiral Stationary Phase For Enantioseparation Of Chiral Amines In Subcritical Fluid Chromatography

Keywords: University of Vienna, Waters, beta blockers, transient buffer species, separation science

Abstract
A new strong cation exchange type chiral stationary phase (SCX CSP) based on a syringic acid amide derivative of trans-(R, R)-2-aminocyclohexanesulfonic acid was applied to subcritical fluid chromatography (SFC) for separation of various chiral basic drugs and their analogues. Mobile phase systems consisting of aliphatic alcohols as polar modifiers and a broad range of amines with different substitution patterns and lipophilicity were employed to evaluate the impact on the SFC retention and selectivity characteristics. The observed results point to the existence of carbonic and carbamic acid salts formed as a consequence of reactions occurring between carbon dioxide, the alcholic modifiers and the amine species present in the sub/supercritical fluid medium, respectively. Evidence is provided that these species are essential for affecting ion exchange between the strongly acidic chiral selector units and the basic analytes, following the well-established stoichiometric displacement mechanisms. Specific trends were observed when different types of amines were used as basic additives. While ammonia gave rise to the formation of the most strongly eluting carbonic and carbamic salt species, simple tertiary amines consistently provided superior levels of enantioselectivity. Furthermore, trends in the chiral SFC separation characteristics were investigated by the systematic variation of the modifier content and temperature. Different effects of additives are interpreted in terms of changes in the relative concentration of the transient ionic species contributing to analyte elution, with ammonia-derived carbamic salts being depleted at elevated temperatures by decomposition. Additionally, in an effort to optimize SFC enantiomer separation conditions for selected analytes, the impact of the type of the organic modifier, temperature, flow rate and active back pressure were also investigated.

http://dx.doi.org/10.1016/j.chroma.2013.03.018

319. Coupling Ultra High-Pressure Liquid Chromatography With Mass Spectrometry: Constraints And Possible Applications
Abstract
The introduction of columns packed with porous sub-2μm particles and the extension of the upper pressure limit of HPLC instrumentation to 1300bar (ultra-high pressure liquid chromatography, UHPLC) has opened new frontiers in resolution and speed of analysis. However, certain constraints appear when coupling UHPLC technology with mass spectrometry (MS). First, the most significant limitation is related to the narrow peaks that are produced by UHPLC that require a fast duty cycle, which is only available on the latest generations of MS devices. Thus, certain analyzers are more readily compatible with UHPLC (e.g., QqQ or TOF/MS) than others (e.g., ion trap or FT-MS). Second, due to the reduction of the column volume, extra-column band broadening can become significant, leading to a reduction in the kinetic performance of the UHPLC-MS configuration. Third, as the mobile phase linear velocity is higher in UHPLC, the electrospray ionization source must also be able to provide high sensitivity at flow rates of up to 1mL/min. Despite these limitations, the UHPLC-MS/MS platform has successfully been employed over the last decade for various types of applications, including those related to bioanalysis, drug metabolism, multi-residue screening, metabolomics, biopharmaceuticals and polar compounds.

http://dx.doi.org/10.1016/j.chroma.2012.09.061

320. Evaluation And Comparison Of Various Separation Techniques For The Analysis Of Closely-Related Compounds Of Pharmaceutical Interest

Abstract
The aim of the present work was to compare various separation techniques for the fast analysis of closely-related compounds, including structurally-related compounds, positional isomers, diastereoisomers, Z/E isomers. Three analytical techniques were evaluated, namely ultra high performance liquid chromatography (UHPLC), ultra high performance supercritical fluid chromatography (UHPSFC), both with sub-2μm particles, and capillary electrophoresis (CE) using non-aqueous solvents. To fairly compare the three analytical techniques, only two starting conditions for further method development were considered. All the selected mobile phases or background electrolyte were MS-compatible. As expected, CE often provided excellent results for the analysis of basic compounds but it was difficult to find out conditions that could be widely applied. On the other hand, UHPLC and UHPSFC were more generic and the performance was better than CE for the analysis of neutral and acidic compounds. In all cases, the analysis time was systematically lower than 3min. In conclusion, UHPLC was the most versatile strategy for the analysis of closely-related compounds and should be tested in a first instance. UHPSFC and CE approaches offered some drastic changes in selectivity and should be considered a second choice to reach alternative selectivity as they also allow high throughput separations.

http://dx.doi.org/10.1016/j.chroma.2013.01.095

321. Analysis Of Food Polyphenols By Ultra High-Performance Liquid Chromatography Coupled To Mass Spectrometry: An Overview

Abstract
Phenolic compounds, which are widely distributed in plant-derived foods, recently attracted much attention due to their health benefits, so their determination in food samples is a topic of increasing interest. In the last few years, the development of chromatographic columns packed with sub-2μm particles and the modern high resolution mass spectrometry (MS) have opened up new possibilities for improving the analytical methods for complex sample matrices, such as ingredients, foods and biological samples. In addition, they have emerged as an ideal tool for profiling complex samples due to its speed, efficiency, sensitivity and selectivity. The present review addresses the use of the improved liquid chromatography (LC), ultra-high performance LC (UHPLC), coupled to MS or tandem MS (MS/MS) as the detector system for the determination of phenolic compounds in food samples. Additionally, the different strategies to extract, quantify the phenolic compounds and to reduce the matrix effect (%ME) are also reviewed. Finally, a briefly outline future trends of UHPLC-MS methods is commented.
322. Efficient Optimization Of Ultra-High Performance Supercritical Fluid Chromatographic Separation Of Rosa Sericea By Response Surface Methodology

2013 - Journal of Separation Science

Keywords: Chengdu Institute of Biology, Shanghai University of Traditional Chinese Medicine, box-behnken design, response surface methodology, natural products, tcm, health science

Abstract

An approach for rapid optimization of ultra-high-performance supercritical fluid chromatographic (UHPSFC) gradient by response surface methodology was developed for fast separation of complex crude extracts of the leaves of Rosa sericea. The optimization was performed with Box–Behnken designs and the multicriteria response variables were described using Derringer's desirability. Based on factorial design experiments, five factors were selected for Box–Behnken designs to optimize the UHPSFC conditions, which led to 46 experiments being performed within 8 h. An evaporative light-scattering detector (ELSD) was used, and quantitative analysis of main components in R. sericea samples was employed to evaluate the statistical significance of the parameters on UHPSFC-ELSD analytes response. The results indicated that the optimized UHPSFC-ELSD method is very sensitive with LODs and LOQs below 1.19 and 4.55 μg/mL, respectively. The overall intra- and interday variations were less than 3.91 and 6.41%, respectively. The recovery of the method ranged from 95.66 to 104.22%, with RSD < 5.91%. This newly developed UHPSFC-ELSD method was demonstrated to be fast and sensitive in analyzing complex herbal extracts of Traditional Chinese Medicines.

http://dx.doi.org/10.1002/jssc.201300289

323. Evaluation Of Various Chromatographic Approaches For The Retention Of Hydrophilic Compounds And MS Compatibility

2013 - Journal of Separation Science

Keywords: University of Geneva, polar compounds, hydrophilic analytes, separation science

Abstract

The goal of this study was to compare the performance of three separation techniques for the analysis of 57 hydrophilic compounds. RPLC, hydrophilic interaction liquid chromatography (HILIC) and subcritical fluid chromatography (SFC) were tested. The comparison was based on the retention, selectivity, peak shape (asymmetry and peak width) and MS sensitivity. As expected, RPLC had some obvious limitations for such classes of compounds, and on average the %ACN required to elute these hydrophilic substances was 4, 7, and 11% ACN at pH 3, 6, and 9, respectively. However, a hybrid polar-embedded C_{18} phase with an appropriate mobile phase could represent a viable strategy for hydrophilic basic compounds with log D greater than −2 on average. HILIC and SFC were found to be more appropriate for analyzing a large majority of these hydrophilic analytes (~60 and 70% of compounds eluted during the gradient in HILIC and SFC), while maintaining good MS sensitivity. Finally, this work demonstrated the complementarity of the three analytical techniques and showed that the selection of a suitable strategy should mostly be based on physicochemical properties of the analytes (pK_a, log D, H-bonding capability, etc.). http://dx.doi.org/10.1002/jssc.201300567

324. 1,4-Naphthoquinones In H-Bond-Directed Trienamine-Mediated Strategies

2013 - Organic Letters

Abstract

The synthesis of optically active, carboannulated dihydronaphthoquinone and naphthoquinone derivatives with up to four stereogenic centers is demonstrated by H-bond-directed, trienamine-mediated [4 + 2]-cycloadditions. The outcome of the reaction between 2,4-dienals and 1,4-naphthoquinones is controlled by the substituent in the 2-position of the 1,4-naphthoquinone. In the case of sterically demanding 2-substituted derivatives, dihydronaphthoquinones are obtained. However, when a hydrogen atom is present in the 2-position, a subsequent oxidation of the initially formed cycloadducts occurs yielding naphthoquinones.

http://dx.doi.org/10.1021/ol401204a
2012 Publications

325. Dienamine-Mediated Inverse-Electron-Demand Hetero-Diels–Alder Reaction By Using An Enantioselective H-Bond-Directing Strategy

By Using An Enantioselective H-Bond-Directing Strategy

Keywords: Aarhus University, asymmetric synthesis, bifunctional catalysis, dienamines, dihydropyran, organocatalysis, separation science

Abstract

Optically active dihydropyran bearing three contiguous stereogenic centers can be efficiently prepared by the title reaction. High stereo- and regiocontrol can be achieved by employing a bifunctional H-bond-directing aminocatalyst.

http://dx.doi.org/10.1002/anie.201207122

326. Analysis Of Basic Compounds By Supercritical Fluid Chromatography: Attempts To Improve Peak Shape And Maintain Mass Spectrometry Compatibility

Attempts To Improve Peak Shape And Maintain Mass Spectrometry Compatibility

Keywords: University of Geneva, basic compounds, 2-ethylpyridine ammonium hydroxide, BEH, GreenSep, Viridis, Zymor Pegasus, PrincetonSFC, pharmaceutical

Abstract

While neutral and acidic compounds are well separated by supercritical fluid chromatography (SFC), basic analytes are more challenging to separate and often problems occur with their peak shapes. Two different methods were explored in the present paper to reduce these problems and maintain compatibility with mass spectrometry (MS). Five different, commercially available 2-ethylpyridine (2-EP) stationary phases were tested without a mobile phase additive using 92 pharmaceutical compounds with basic properties. The kinetic performances of the 5 columns were nearly identical, but the peak shapes of the basic drugs were strongly affected by the stationary phase. The PrincetonSFC 2-EP and Zymor Pegasus 2-EP phases clearly outperformed the other stationary phases, with 77% and 69% of the compounds having Gaussian peaks (and asymmetries between 0.8 and 1.4), respectively. Comparatively, the Waters Viridis Silica 2-EP, Waters Viridis BEH 2-EP and ES Industries GreenSep 2-EP phases provided only 52%, 44% and 22% of the compounds with Gaussian peaks, respectively. These differences were attributed to the significant dissimilarities in their silica matrix properties. An alternative strategy was also performed with a hybrid silica stationary phase, Viridis BEH, using 20mM ammonium hydroxide in the mobile phase, which was a mixture of CO(2) and MeOH. With these conditions, 81% of the peaks observed for the basic analytes were Gaussian; however, this value dropped to 17% and 10% in the absence of additive and in the presence of 20mM formic acid, respectively. Finally, the use of a hybrid bare silica stationary phase in the presence of 20mM ammonium hydroxide is quite an interesting solution as this system is compatible with both ultra high performance SFC (UHPSFC) columns packed with sub-2 μm particles and with MS detection. The overall applicability of this system was demonstrated with various mixtures of basic drugs.

http://dx.doi.org/10.1016/j.chroma.2012.08.091

327. Comparison Of Ultra-High Performance Supercritical Fluid Chromatography And Ultra-High Performance Liquid Chromatography

Comparison Of Ultra-High Performance Supercritical Fluid Chromatography And Ultra-High Performance Liquid Chromatography

Keywords: University of Geneva, acidic, neutral, basic, backpressure, frictional heating, separation science
Abstract
Currently, columns packed with sub-2 μm particles are widely employed in liquid chromatography but are scarcely used in supercritical fluid chromatography. The goal of the present study was to compare the performance, possibilities and limitations of both ultra-high performance liquid chromatography (UHPLC) and ultra-high performance supercritical fluid chromatography (UHPSFC) using columns packed with sub-2 μm particles. For this purpose, a kinetic evaluation was first performed, and van Deemter curves and pressure plots were constructed and compared for columns packed with hybrid silica stationary phases composed of 1.7 and 3.5 μm particles. As expected, the kinetic performance of the UHPSFC method was significantly better than that of the UHPLC. Indeed, the h(min) values were in the same range with both strategies and were between 2.2 and 2.8, but u(opt) was increased by a factor of >4 in UHPSFC conditions. Another obvious advantage of UHPSFC over UHPLC is related to the generated backpressure, which is significantly lower in the presence of a supercritical or subcritical fluid. However, the upper pressure limit of the UHPSFC system was only ~400 bar vs. ~1000 bar in the UHPLC system, which prevents the use of highly organic mobile phases at high flow rates in UHPSFC. Second, the impact of reducing the particle size (from 3.5 to 1.7 μm) was evaluated in both UHPLC and UHPSFC conditions. The effect of frictional heating on the selectivity was demonstrated in UHPLC and that of fluid density or decompression cooling was highlighted in UHPSFC. However, in both cases, a change in selectivity was observed for only a limited number of compounds. Third, various types of column chemistries packed with 1.7 μm particles were evaluated in both UHPLC and UHPSFC conditions using a model mixture of acidic, neutral and basic compounds. It has been shown that more drastic changes in selectivity were obtained using UHPSFC columns compared to those obtained by changing UHPLC columns. In addition, there was a good complementarity between the two separation modes. Finally, by combining the use of small particles with supercritical fluids as a mobile phase, it was possible to achieve the analysis of pharmaceutical compounds in less than 1 min or to attain a peak capacity of more than 250 in approximately 40 min, both with a high degree of repeatability.

http://dx.doi.org/10.1016/j.chroma.2012.10.005

328. Enantioselective Formation Of Substituted 3,4-Dihydrocoumarins By A Multicatalytic One-Pot Process

Keywords: dihydrocoumarins, aminocatalysis, redox reaction monitoring

Abstract
The formation of optically active 3,4-dihydrocoumarins is presented by merging aminocatalysis with an N-heterocyclic carbene-catalyzed internal redox reaction. The products are formed in good to excellent yields and in general with excellent enantioselectivities. Moreover, the developed procedure demonstrates the potential of enantioselective, multicatalytic sequences. By employing an enantiopure aminocatalyst in the enantiodifferentiating step, the challenges related to achieving high stereoiductions by deployment of optically active NHC-catalysts can be circumvented.

http://dx.doi.org/10.1021/ol302627u