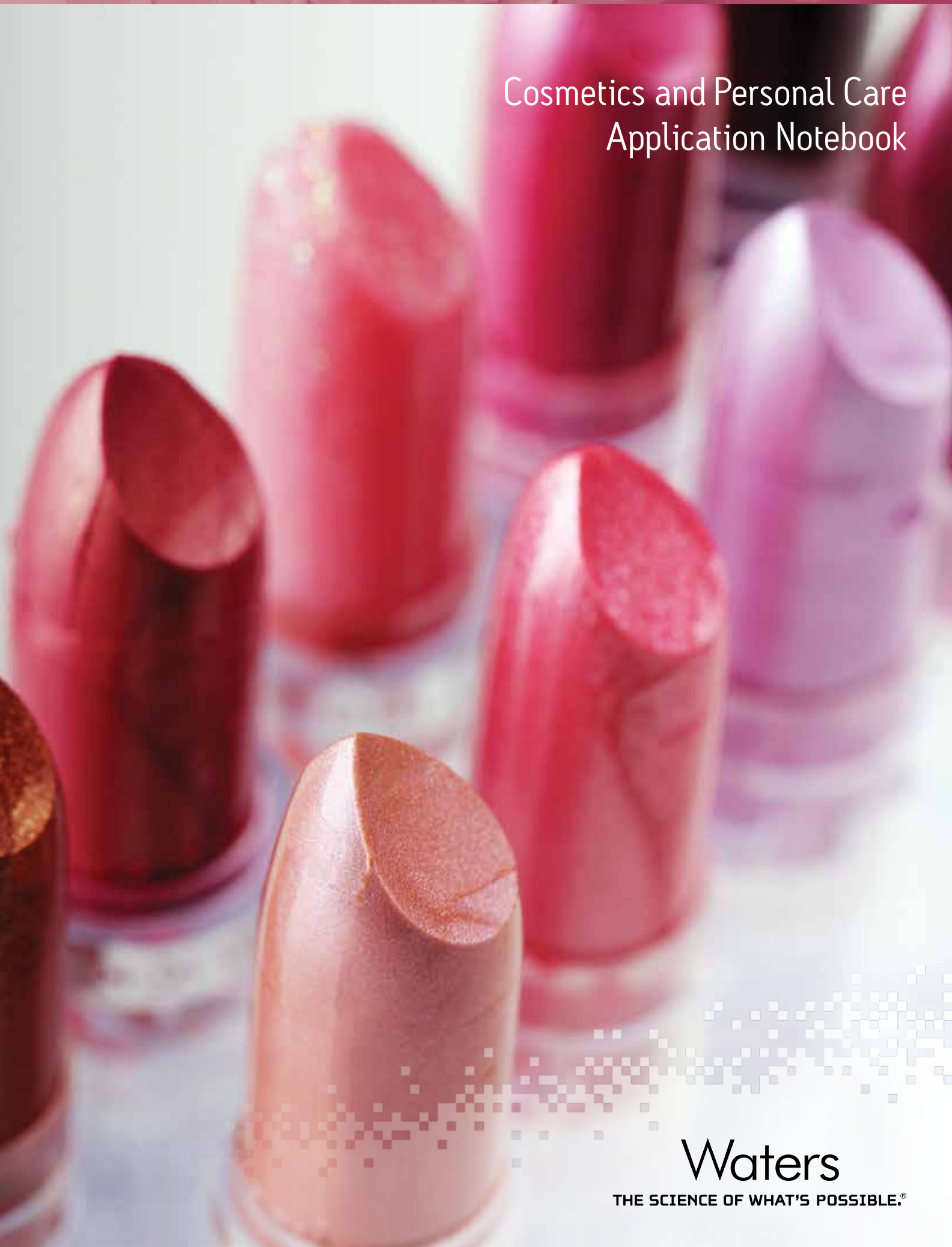
Cosmetics and Personal Care Application Notebook



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For years, Waters Corporation has provided the top cosmetic and personal care companies in the world with an innovative suite of technologies designed to improve the effectiveness of raw material, formulation, safety, and quality control testing. Our continuous advancements in sample preparation and columns, chromatography, mass spectrometry, and data management software enable analytical laboratories to reduce analysis times, improve data quality, lower costs, and meet compliance requirements in an ever changing regulatory environment.

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RAW MATERIAL TESTING

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Analysis of the Non-Ionic Surfactant Triton-X Using UltraPerformance Convergence Chromatography (UPC²) with MS and UV Detection

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APPLICATION BENEFITS

UPC²® with either UV or MS detection for the analysis of non-ionic surfactant, offers:

- High-efficiency separation with excellent resolution for approximately 20 oligomers.
- Analysis time less than 2 min with PDA detection.
- Reduction in consumption of organic solvents.
- Analysis at lower temperatures than in GC or SFC.
- The detection of: additional minor series components; by-products; impurities; degradation products or contaminants.

WATERS SOLUTIONS

ACQUITY UPC²® System

Xevo® TQD

MassLynx® Software

ACQUITY UPC² PDA Detector

Empower® 3 Software

KEY WORDS

Triton-X, cosmetics, personal care products, household and industrial cleaning products

INTRODUCTION

The non-ionic surfactant Triton X-100 (Figure 1), an excellent detergent and wetting agent, is readily biodegradable and achieves effective performance across a broad temperature range. It can also be used as a dispersant and emulsifier for oil in water systems. Because of these properties, Triton X-100 is used in many household and industrial cleaning products, paints and coatings, pulp and paper, oil fields, textiles, agrochemicals, cosmetics, and industrial materials.

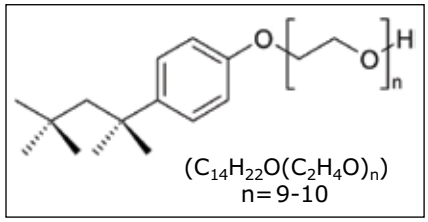

Figure 1. Triton-X-100 structure and chemical formula.

It is essential to be able to monitor the composition of the non-ionic, octylphenol ethoxylate surfactant Triton X-100, because differences in the ethoxy chain length can affect characteristics of the mixture such as viscosity, solubility, and polarity.

The ability to detect the presence of by-products, impurities, degradation products or contaminants present in surfactants is equally important. In addition to identifying potential carcinogenic or allergenic compounds, the presence of impurities can also affect the efficiency of the surfactant.

Surfactants are typically analyzed using techniques such as High Performance Liquid Chromatography (HPLC),[1,2] Supercritical Fluid Chromatography (SFC),[3] or Gas Chromatography (GC).[4,5] Analysis by GC and HPLC can be time consuming, as these techniques may require additional derivatization stages in order to improve sensitivity, separation or resolve volatilization issues. GC or traditional SFC techniques that employ high column temperatures can also limit the analysis of thermally labile compounds. In some cases, baseline separations for oligomers using HPLC, SFC or GC analyses are not achieved.

Waters® UltraPerformance Convergence Chromatography™ (UPC^2) System, builds on the potential of normal-phase separation techniques such as SFC, while using proven Waters' easy-to-use UPLC® Technology.

This application note describes the analysis Triton X-100 utilizing UPC^2 with PDA and MS detection. Excellent resolution for approximately 20 oligomers has been achieved using lower temperatures than GC or traditional SFC analysis, making UPC^2 more amenable for the analysis of thermally labile compounds. A significant reduction in the consumption of toxic solvents was also achieved compared to normal phase HPLC analysis.

EXPERIMENTAL

UV conditions

UV system:	ACQUITY UPC^2 PDA Detector
Range:	210 to 400 nm
Resolution:	4.8 nm
UPC^2 System:	ACQUITY UPC^2
Column:	ACQUITY UPC^2 BEH 2.1 mm x 50 mm, 1.7 µm
Column temp.:	40 °C
Convergence column manager back pressure:	1500 psi
Injection volume:	1.0 µL
Mobile phase B:	Methanol

Mobile phase gradient for UV detection is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	2.00	98.0	2.0	—
2	1.25	2.00	65.0	35.0	6
3	1.30	2.00	98.0	2.0	6
4	2.00	2.00	98.0	2.0	6

Table 1. ACQUITY UPC^2 mobile phase gradient for UV detection.

Instrument control, data acquisition, and result processing

Empower 3 Software was used to control the ACQUITY UPC^2 System and ACQUITY UPC^2 PDA Detector, and provide data acquisition and processing.

MassLynx Software was used to control the ACQUITY UPC^2 System and Xevo TQD, and provide data acquisition and processing.

MS conditions

MS system:	Xevo TQD
Ionization mode:	ESI +
Capillary voltage:	3.5 kV
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas flow:	800 L/hr
Cone gas flow:	50 L/hr
Acquisition:	Full scan
UPC^2 System:	ACQUITY UPC^2
Column:	ACQUITY UPC^2 BEH 2.1 mm x 50 mm, 1.7 µm
Column temp.:	65 °C
CCM back pressure:	1600 psi
Injection volume:	1.0 µL
Mobile phase B:	Methanol

Mobile phase gradient for MS detection is detailed in Table 2.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	2.00	97.0	3.0	–
2	20.00	2.00	80.0	20.0	6
3	21.00	2.00	97.0	3.0	6
4	23.00	2.00	97.0	3.0	6

Table 2. ACQUITY UPC^2 mobile phase gradient for MS detection.

RESULTS AND DISCUSSION

UV detection results

UPC^2 conditions were optimized for the separation and detection of 20 Triton X-100 oligomers. The UV chromatogram for a 10 mg/mL standard in isopropanol alcohol is shown in Figure 2.

MS detection results

The UV method demonstrated the speed and simplicity of UPC^2 for the analysis of Triton X-100. With further optimization of the separation, in this example using a slower gradient, with MS detection additional characterization of the surfactant was achieved.

The chromatogram for Triton X-100 with MS detection, using the described UPC^2 and MS conditions, is shown in Figure 3. The oligomers detected can be further identified considering the MS spectra, shown in Figure 4 for the oligomers identified as a, b, c, and d in Figure 3.

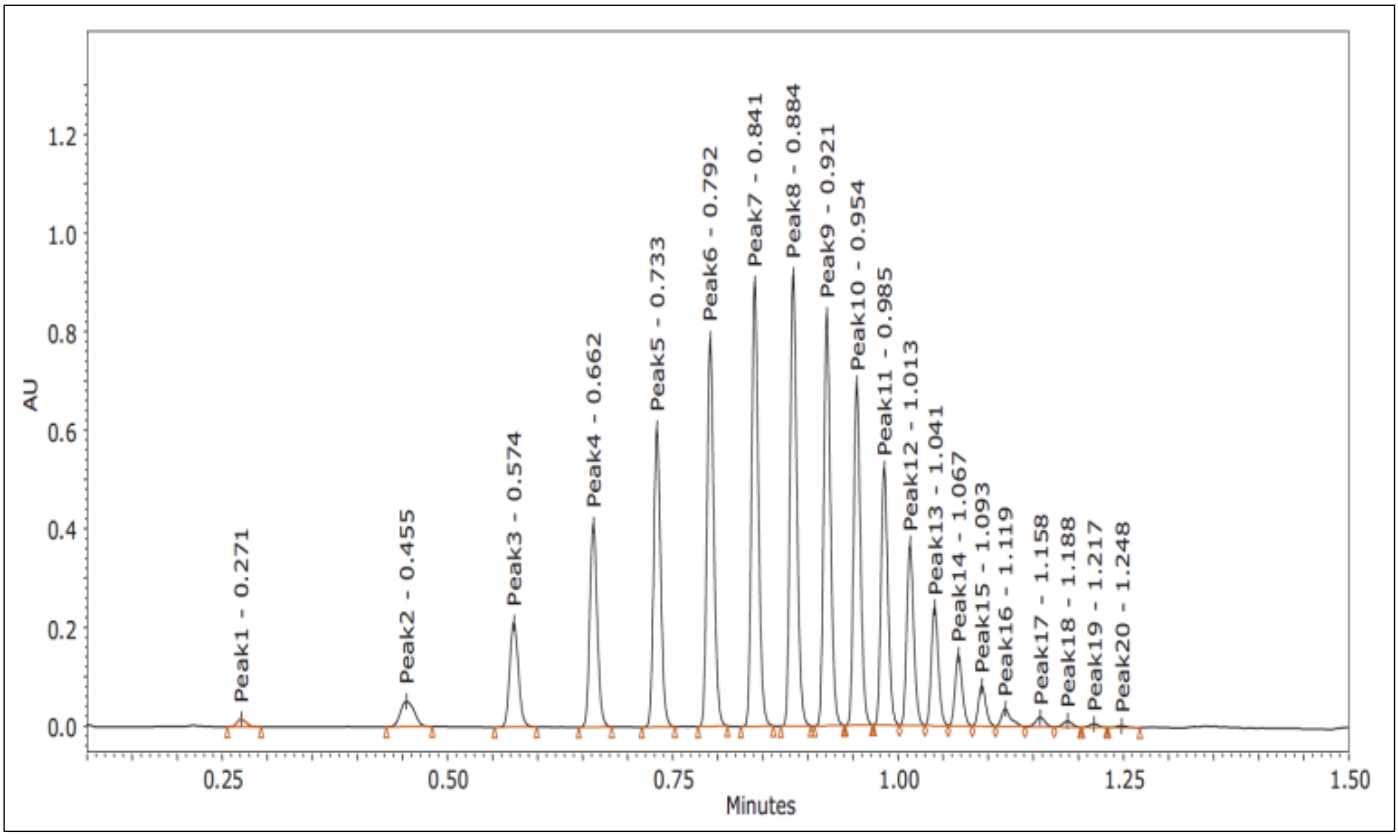

Figure 2. UV chromatogram for a 10 mg/mL Triton X-100 standard.

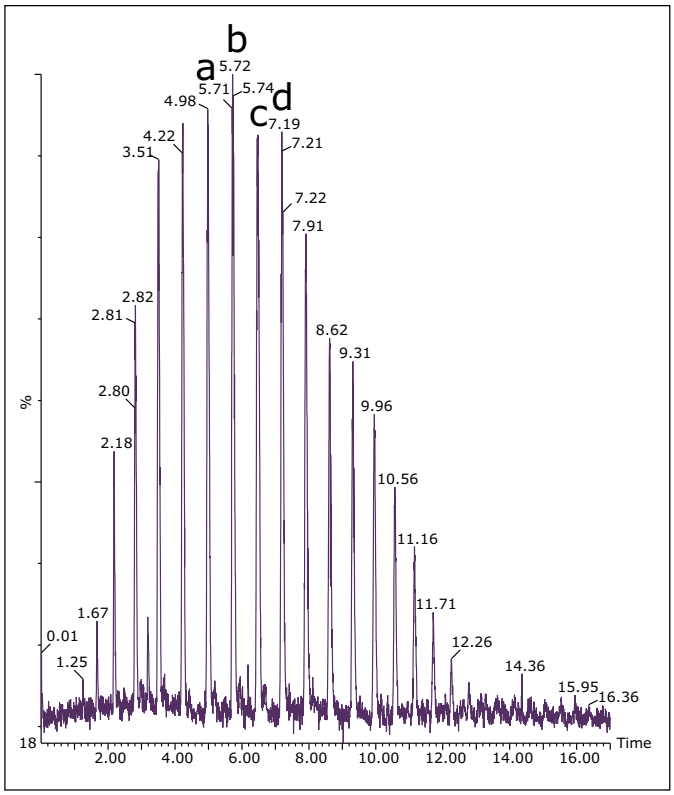

Figure 3. MS chromatogram for a Triton X-100 standard.

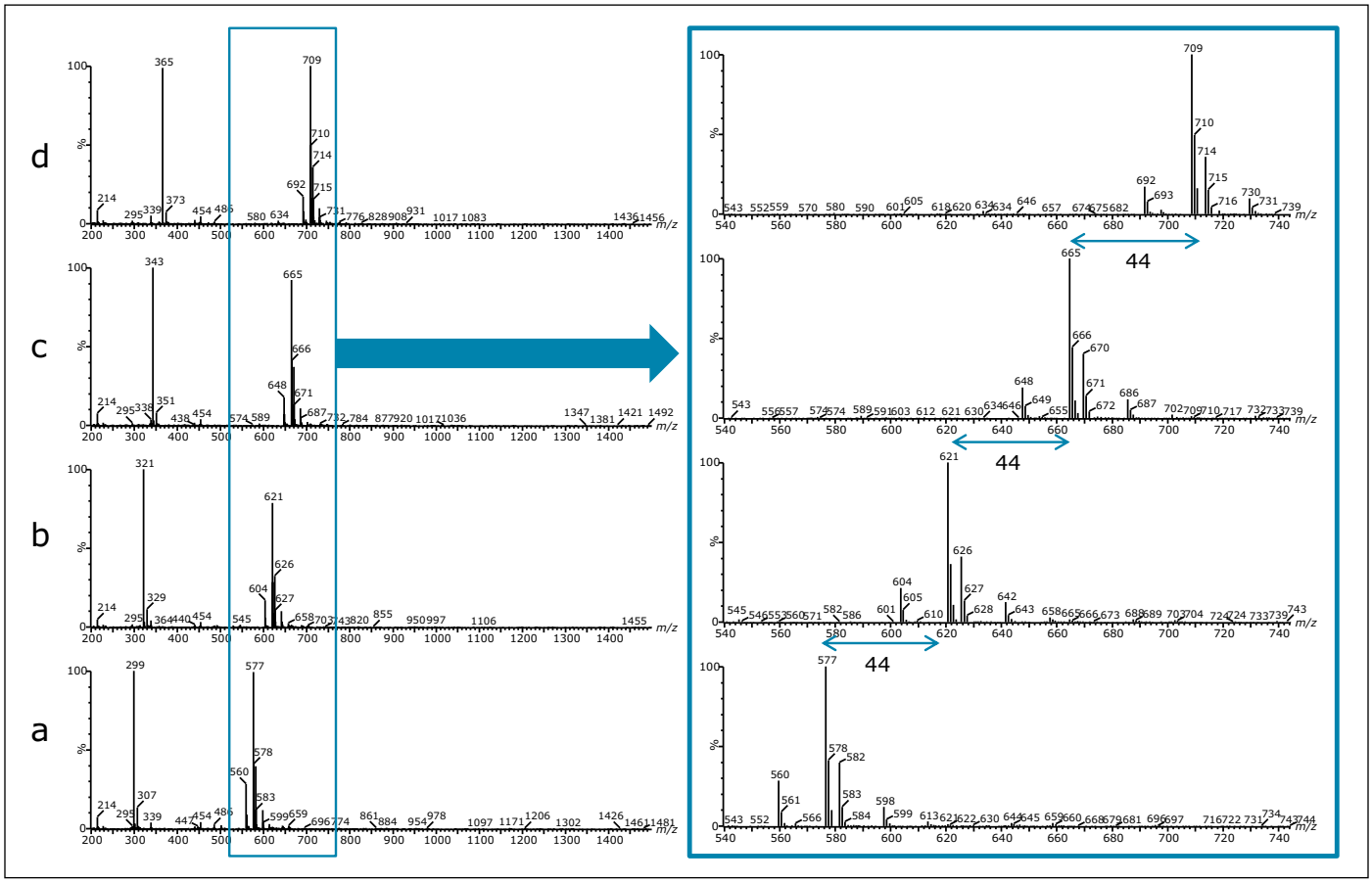

Figure 4. Mass spectra for the individual Triton-X oligomers as indicated in Figure 3.

By using a slower gradient additional details can be observed, such as the detection of: additional minor series components, by-products, impurities, degradation products, or contaminants. An additional minor series present in the analyzed sample of Triton X-100 is shown in Figure 5.

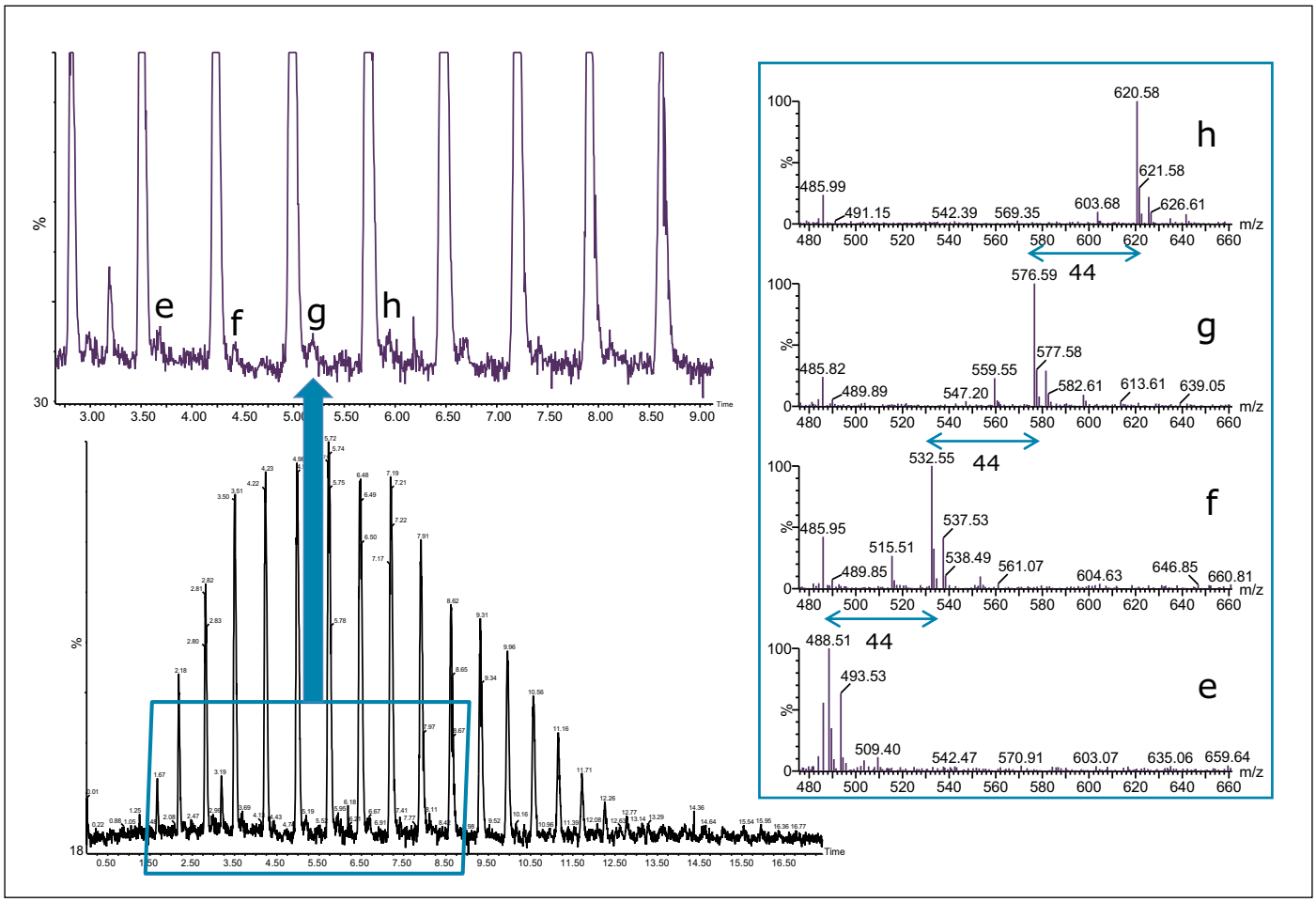

Figure 5. Additional minor series highlighted in the analyzed sample of Triton X-100, with respective mass spectra.

CONCLUSIONS

- Rapid, high efficiency separation with analysis time of less than 2 min with PDA detection.
- Excellent resolution for approximately 20 oligomers.
- Analysis occurs at lower temperature than in GC or SFC.
- Reduction in consumption of organic solvents.
- MS detection can be used to further characterize the surfactant, such as the identification of specific oligomers, detection of additional series components, by-products, impurities, degradation products of contaminants.

References

1. R A Escott, S J Brinkworth, T A Steedman. The determination of ethoxylate oligomers distribution of Non-Ionic and Anionic Surfactants by HPLC. *J Chromatography*. 282: 655–661, 1983.
2. K Nakamura, Y Morikawa, I Matsumoto. Rapid Analsyis of Ionic and Non-Ionic Surfactants Homologs by HPLC. *Journal of the American Oil Chemists' Society*. 58: 72–77, 1981.
3. B J Hoffman, L T Taylor. A Study of Polyethoxylated Alkylphenols by Packed Column Supercritical Fluid Chromatography. *Journal of Chromatography*. 40: 61–68; Feb 2002.
4. C Bor Fuh, M Lai, H Y Tsai, C M Chang. Impurity analysis of 1,4-dioxane in nonionic surfactants and cosmetics using headspace solid-phase microextraction coupled with gas chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography A*. 1071: 141–145; 2005.
5. J A Field, D J Miller, T M Field, S B Hawthorne, W Giger. Quantitative determination of sulfonated aliphatic and aromatic surfactants in sewage sludge by ion-pair/supercritical fluid extraction and derivatization gas chromatography/mass spectrometry. *Analytical Chemistry*. 64(24): 3161–3167; 1992.

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Characterizing of the Natural Product Goldenseal Using CORTECS 2.7 µm Columns and ACQUITY QDa Detection

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APPLICATION BENEFITS

- Separation of complex sample matrices using CORTECS® 2.7 µm Columns, allowing for accurate characterization of the sample
- Rapidly identify compounds by mass using the ACQUITY® QDa® Detector in 5 minutes

WATERS SOLUTIONS

CORTECS C_{18}+ Columns

Alliance® HPLC

Empower® 3 CDS

LCMS Certified Max Recovery Vials

ACQUITY QDa Detector

KEY WORDS

Goldenseal, cosmetics, personal care products, natural products

INTRODUCTION

Natural product herbal supplements are widely used as remedies for physical ailments. Depending on sample origin and processing, product composition can range widely in the number, type, and amount of natural product compounds present. As consumers are growing in concern to the side effects of chemicals used in cosmetics and personal care products, the use of herbal plants in cosmetics,[1] and personal care products,[1,2] is growing in demand. Accurate sample characterization from numerous sources is useful to control supplement quality. However, the sample complexity and/or variability require highly efficient columns that do not sacrifice analysis speed. CORTECS 2.7 µm Columns contain solid-core particles which produce high peak capacity separations with reduced back pressure, making them ideal for use on traditional HPLC instruments. Using CORTECS 2.7 µm Columns, therefore, allows easier analysis of natural product mixtures.

Goldenseal is a plant native to southeastern Canada and the northeastern United States. Traditionally, it has been used to support digestion, mucous membranes, bile secretions, and many other bodily functions, as well as a topical treatment. In this application note, five different sources of Goldenseal which span four different manufacturers, two different parts of the plant, and two different formulation matrices are analyzed. This variety of sources was tested to determine the differences between each sample and if there were any compounds common to all the tested sources. Each sample was analyzed using a CORTECS C_{18}+, 2.7 µm, 3.0 x 50 mm Column on an Alliance HPLC System with both UV and an ACQUITY QDa Detector in order to get fast and reliable mass data for peaks present in the sample, providing additional and crucial information for the characterization of the natural products.

EXPERIMENTAL

LC conditions

System:	Alliance HPLC
Column:	CORTECS C_{18}+, 2.7 µm, 3.0 x 50 mm (p/n 186007400)
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	7–30% B in 5.0 minutes, return to 7% B in 0.1 minutes, hold for 1.0 minutes
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Detection (UV):	300 nm
ACQUITY QDa setting:	ESI+ mode, full scan from 150–1250 amu
Injection volume:	1.0 µL
Sample vials:	LCMS Certified Max Recovery Vials (p/n 600000670CV)
Data management:	Empower 3 CDS

Sample preparation

Capsule samples[3]

20 mg of powdered sample was removed and placed into a 10 mL centrifuge tube. 2.5 mL of 90:10 methanol:water with 0.1% acetic acid was added. Samples were sonicated for 15 minutes, and centrifuged at 4000 rpm for 5 minutes. The supernatant was then removed and placed into a separate vial. Extraction was performed three additional times. Extracted liquid filtered through a 0.1 µm nylon filter prior to injection.

Liquid sample

Two drops of liquid Goldenseal was added to 10 mL of 90:10 methanol:water with 0.1% acetic acid. Sample filtered through a 0.1 µm nylon filter prior to injection.

RESULTS AND DISCUSSION

Five commercial sources of Goldenseal were acquired for characterization. Table 1 outlines the source of each sample as well as details regarding the part of the plant used and the sample format.

Name	Source	Format
Goldenseal liquid	Root	Liquid
Goldenseal herb	Stem, flower, leaf	Capsule
Goldenseal root	Root	Capsule
Goldenseal extract	Root	Capsule
Goldenseal	Rhizome/root	Capsule

Table 1. Summary of the five sources of Goldenseal obtained.

After sample preparation, the samples were injected and both UV and mass data were collected. Figure 1 shows the full scale separation of the five samples.

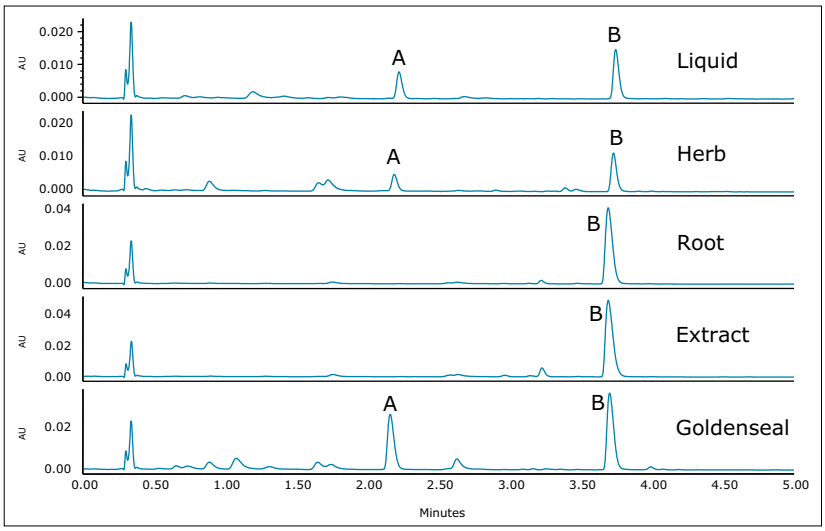

Figure 1. Separation of five different sources of Goldenseal on a CORTECS C_{18}+, 2.7 µm, 3.0 x 50 mm Column at 300 nm. Main components A and B are indicated. Maximum pressure for the separation was 2600 psi.

The chromatograms were then scaled to show the low level constituents that exist in the samples. Figure 2 shows the zoomed in chromatograms of the five samples.

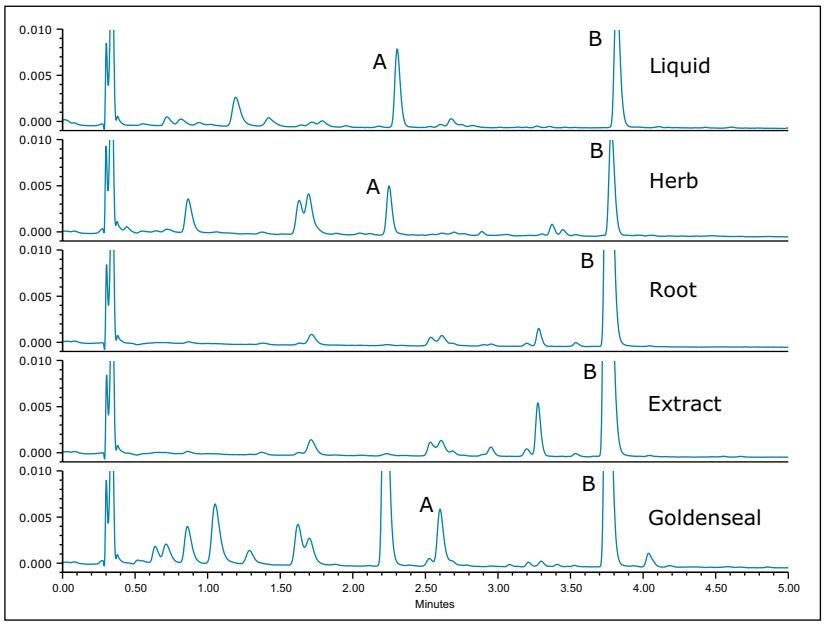

Figure 2. Zoomed in UV chromatograms of the five samples from Figure 1.

The distinct differences in the UV chromatograms of the five different samples were evident due to the high efficiency of the CORTECS Column. The intensity of peak B varied from 0.04 to 0.02 AU, indicating that different samples had different amounts of that particular compound. Further sample characterization required additional information. To obtain this, ACQUITY QDa mass spectral data was examined and compared with Goldenseal data from the literature.[4] A total of seven alkaloids were identified (see Table 2 and Figure 3).

Compound	Mass (M+H)
Dihydro Berberine	338.1
Canadine	340.3
Berberine	336.1
Isocorypalmine	370.1
Methyl Hydrastine	398.4
Hydrastine	384.1
Palmatine	352.2

Table 2. Summary of alkaloids identified and their masses as described in the literature.[4]

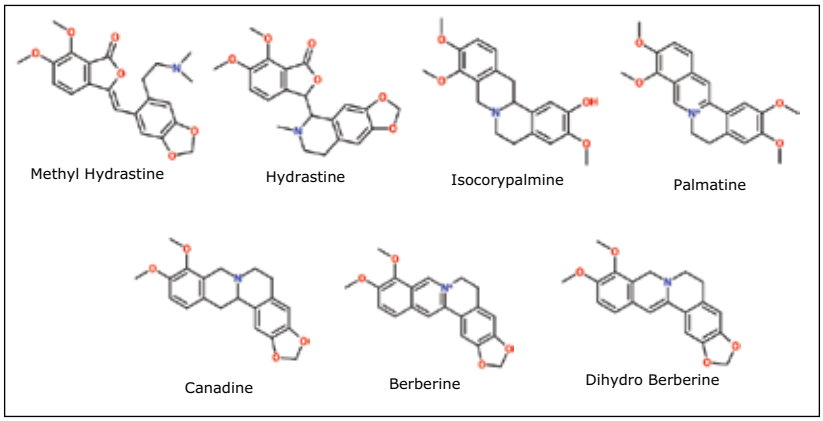

Figure 3. Structures of identified alkaloids.

Using the ACQUITY QDa Detector, some of the peaks observed in the UV chromatograms were identified. The liquid sample had the most identifiable peaks, showing the presence of all seven compounds listed in Table 2. The remaining samples each exhibited two or more identifiable peaks. Berberine was the most abundant component and the only compound present in all samples. Figure 4 shows the identification of peaks in the liquid sample using extracted ion chromatograms (EIC).

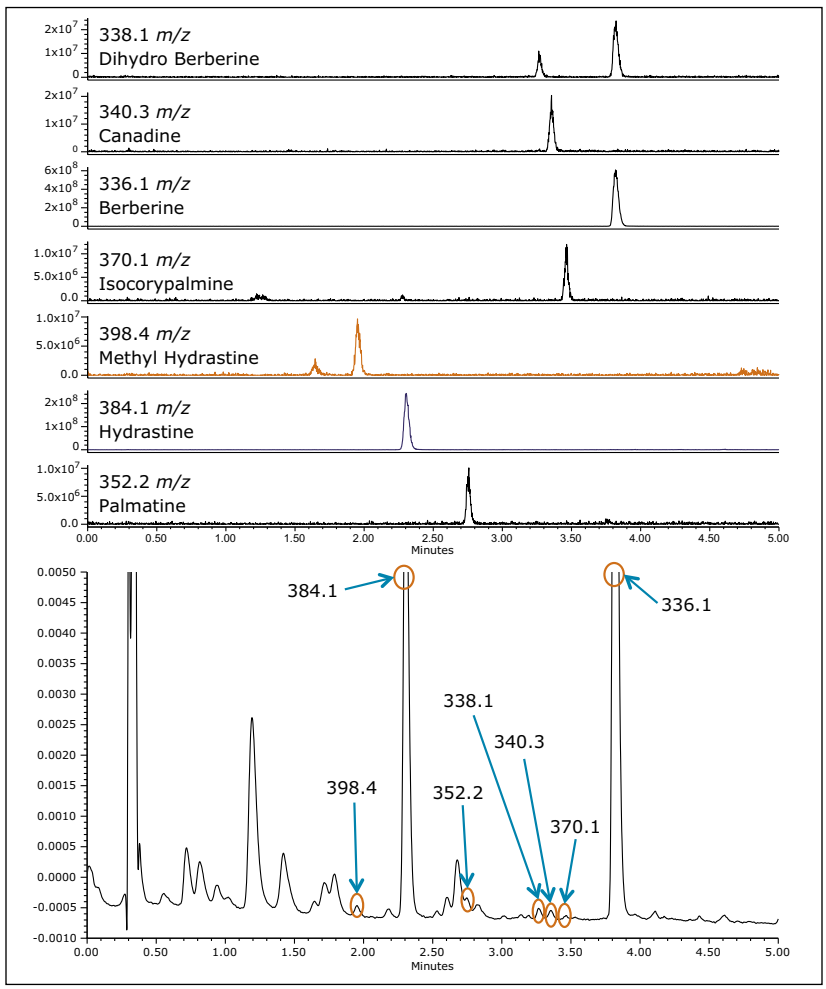

Figure 4. Identification of UV peaks in the liquid sample of Goldenseal by m/z value.

By using the ACQUITY QDa Detector, the two main component peaks (A and B in Figures 1 and 2) were identified as hydrastine and berberine, respectively. An additional five compounds were also identified and linked to peaks in the UV chromatogram. No two samples show exactly the same compounds at the same concentrations. These traces represent a type of "fingerprint" that is characteristic of each Goldenseal sample. Such fingerprints can be useful in comparing Goldenseal from different manufacturers as well as different sources of the plant. The rapid separation of a complex sample such as Goldenseal is possible due to high efficiency of CORTECS 2.7 µm Columns. By combining UV data with the mass data obtained with an ACQUITY QDa Detector, a full characterization of each sample can be made giving an analyst valuable information with minimal effort.

CONCLUSIONS

Natural product analysis and characterization can be a difficult process due to the complex nature of the sample. Gathering fast and reliable data is essential for characterization of complex samples such as Goldenseal in this application. Using a CORTECS C_{18}+ 2.7 µm Column, a complex separation can be performed more easily. CORTECS 2.7 µm Columns offer high efficiency while operating within the pressure limits of an HPLC system. By combining the newest column technology with the newest technology in mass detection (ACQUITY QDa), a simple separation of five sources of Goldenseal was performed in five minutes and seven compounds were identified.

References

1. S K Gediya, R B Misty, U K Patel, et al. Herbal Plants: Used as a Cosmetics. *Journal of Natural Product and Plant Resources.* 2011; 1(1):24-32.
2. V P Kapoor. Herbal Cosmetics for Skin and Hair care. *Natural Product Radiance.* 2005; 4(4): 306-314.
3. Avula, B *et al*. Quantitative Determination of Alkaloids from Roots of *Hydrastis canadensis L*. and Dietary Supplements Using UltraPerformance Liquid Chromatography with UV Detection. *Journal of AOAC International.* Vol 95 (2012) 5:1398–1405.
4. Pillai, M *et al*. LC-MS Based Workflows for Qualitative and Quantitative Analysis of Homeopathic Preparation of *Hydrastis canadensis*. *Chromatographia* (2014) 77: 119–131.

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Using the Elucidation Tool in UNIFI Scientific Information System to Identify Unknown Compounds in Natural Products

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GOAL

To identify components from a sample that either were not matched with scientific libraries within the UNIFI® Scientific Information System, or were matched but were suspected to be false positive, the Elucidation Tool within UNIFI can be used. An example is used to demonstrate the process using the Natural Product Application Solution with UNIFI.

BACKGROUND

Several scientific libraries are integrated within workflows in the UNIFI Scientific Information System, providing convenience and support for component identification of unknown samples. For components successfully matched with a library, researchers only need to verify the rationality of the fragments that have been classified by MassFragment™ Software to confirm the target components.

However, for components that cannot be matched with a UNIFI library, or that can be matched but false positives are suspected, UNIFI's Elucidation Tool can be used to manually identify the target compounds of interest through searching online libraries. Here, using the Natural Products Application Solution with UNIFI, we illustrate this process by investigating the identification of an unknown component in a natural product extract as an example.

The Elucidation Tool is a standard feature within the UNIFI Scientific Information System that facilitates the identification of unknown compounds. This feature combines compound identification, by searching online libraries based on elemental composition, with structural elucidation using MassFragment and its MS fragmentation data.

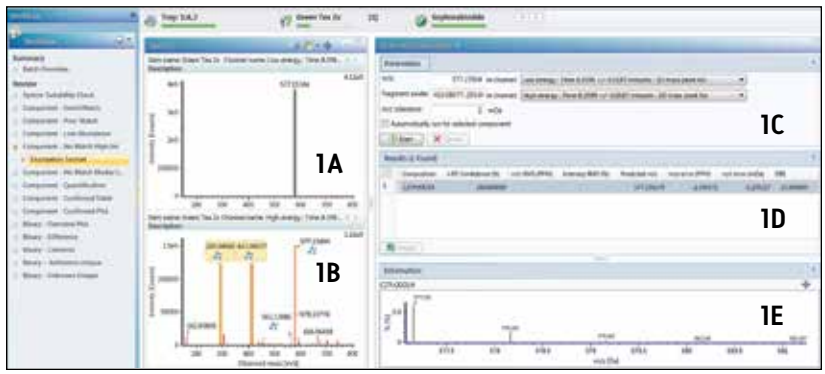

Figure 1. The process of an unknown component's elemental composition determination. 1A: Low collision energy mass spectrum of the unknown component. 1B: High collision energy mass spectrum of the unknown component. 1C: Settings for elemental composition search. 1D: Search results of the unknown component's elemental composition. 1E: Isotope distribution plot for the unknown sample.

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THE SOLUTION

The processes and steps for unknown compound identification using the Elucidation Tool are shown in Figures 1 and 2.

The first step is to determine the elemental composition of the unknown component. In UNIFI, the elemental composition of an unknown compound is determined by three combined factors: exact mass of the intact precursor ion, exact mass and abundance ratio of isotopic peaks, as well as confirming elemental composition of the secondary fragment ions that are correspond to precursor ions.

As shown in Figure 1, for the unknown component with an accurate mass of 577.1550, we can obtain the only possible elemental composition combining the above three factors: $C_{27}H_{30}O_{14}$ (assuming this natural product is only composed of C, H, and O).

If the elemental composition corresponding to the secondary fragments is not taken into consideration, three possible molecular formulas could be obtained when searching the elemental composition of this unknown component, and manual evaluation is then required. However, because the fragmentation ions are taken into consideration, the false positives were excluded, leading to a single elemental composition as the accurate and reliable result.

The second step in this identification process is to search possible names and associated structures of chemical ingredients through online libraries. UNIFI Software links directly to ChemSpider, enabling researchers to search online and obtain possible structures in a variety of ways. For example, one can select all 558 UNIFI default libraries, or simply select some associated libraries. Alternatively, one can conduct the online search by either elemental composition or by accurate mass. Figure 2 shows the process of searching the online libraries.

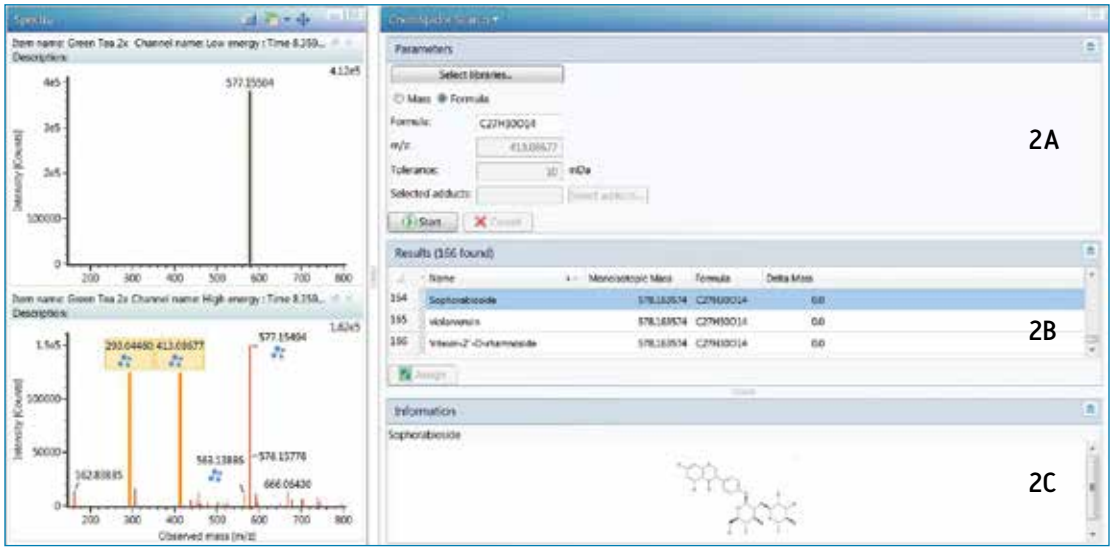

Figure 2. UNIFI directly links to ChemSpider for online library searches. 2A: Parameter settings for ChemSpider online search. 2B: Matching list as the result of the online search;. 2C: Chemical structures corresponding to the results list. This figure shows the preliminarily confirmed compound structures after screening.

After the confirmation of the name and structure of the target compound, clicking Assign will allow this result to be directly brought into the Component Summary List, which resides within the Review tab, and consequently changes the target compound's identification status from Unknown to Identified.

To further confirm the authenticity and reliability of this matched compound, one can utilize MassFragment and manually match fragment ions based on the proposed matching structure for the compound. In this example, we use the fragment ions from the high collision energy scan along with the fragment matching function in the Elucidation Tool. In Figure 3, a fragment ion is used to demonstrate this process and to show how fragmentation pathway can be determined. The most reasonable choice can be manually confirmed from all possible structures matched with this fragment. When clicking the Assign button, this fragment structure is linked to the associated fragment peak in high collision energy scan spectrum, and displayed by a blue icon.

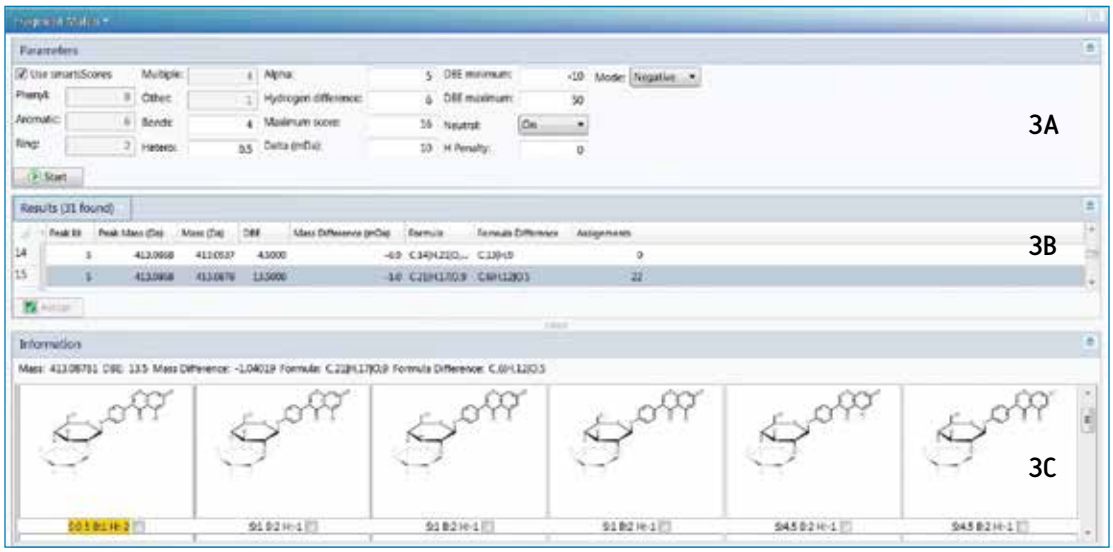

Figure 3. Manual fragment matching interface. 3A. Parameter settings for fragment matching; 3B. List of fragment matching results; 3C. Fragment structure diagram.

Upon confirmation of the key fragment ions, one can further label the target compound as Confirmed from the Component Summary List that resides within the Review tab. If required, one can indicate its database source in the Comment column, as seen in Figure 4. Finally, all Confirmed results are shown in the Components-Confirmed Table workflow.

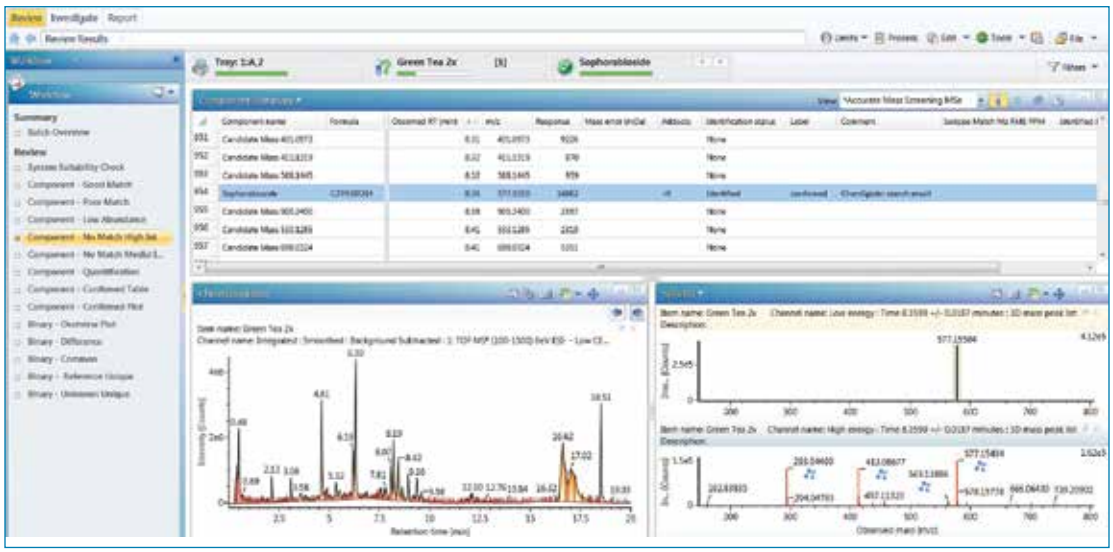

Figure 4. The final identification result of the unknown sample is shown in Compound Summary List in the Review interface.

SUMMARY

This work describes how to use the Elucidation Tool within the UNIFI Scientific Information System to manually identify unmatched or matched but suspected false-positive components. This process has a rational design, the elemental composition search is reliable and accurate, and the ability to use online library searches is simple and intuitive. The target compound can be further confirmed utilizing MassFragment Software. In this example, by using the Natural Product Application Solution with UNIFI, the unknown compound that elutes at 8.35 min with [M-H]- of 577.1550 is confirmed to be Sophrabioside.

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Improving the Productivity in Isolating a Naturally Occurring Bioactive Compound Using Supercritical Fluid Extraction and Preparative Supercritical Fluid Chromatography

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Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- SFE alleviates the sample complexity in natural product extracts prior to chromatographic analysis and purification, enabling a more efficient purification downstream.
- SFC offers complementary separation to RPLC. In addition, there is a wide range of column chemistries available in SFC with vastly different separation mechanisms. The combinations of SFC/RPLC and SFC/SFC provide unmatched resolving power to meet the challenges, primarily arising from sample complexity, in natural product isolation.
- Both SFE and SFC reduce the use of organic solvents and provide an easy sample recovery under mild conditions, thereby increasing the overall purification productivity and cost-effectiveness.

WATERS SOLUTIONS

ACQUITY UPC²® System

ACQUITY UPLC® H-Class System

AutoPurification™ LC System

Prep 100q SFC MS Directed System

ACQUITY UPC² BEH 2-EP Column

Viridis® Silica 2-EP Column

ChromScope™ Software

KEY WORDS

Natural product, purification, prep chromatography, SFE, SFC, UPC², cosmetics, selectivity, productivity, orthogonality

INTRODUCTION

Natural products are a productive source of leads for new drugs due to their high chemical diversity, biochemical specificity, and many "drug-likeness" molecular properties.[1-4] A large portion of today's existing drugs on the market are either directly derived from naturally occurring compounds or inspired by a natural product. In addition, natural products are also used in the forms of food supplements, nutraceuticals, alternative medicines,and as active ingredients in cosmetics.[5]

Isolation and purification of bioactive compounds play an important role in natural product research. The most commonly used process often involves extraction of target compounds from the cellular matrix, pre-purification by various chromatographic techniques including flash chromatography (FC), low pressure liquid chromatography (LPLC), and medium pressure liquid chromatography (MPLC), followed by preparative high pressure liquid chromatography (prep HPLC).[6] However, this process is not without its challenges. For example, conventional extraction methods for natural products include Soxhlet extraction, maceration, percolation, and sonication. These methods are often time- and labor-intensive, consume large amounts of organic solvents, and can lead to the degradation of thermally labile compounds. Furthermore, prep chromatography is largely dominated by reversed-phase liquid chromatography (RPLC), whereby the separation is driven by the differentiating polarity of the analytes. While a generally applicable chromatographic technique for a variety of compound classes, RPLC does not necessarily guarantee an adequate resolution for all analytes, especially for the structural analogs and isomers of similar polarities often found in natural products. As a result, the purification step is perceived by many as a rate-limiting step and a major bottleneck for natural product drug discovery, as well as in the development of differentiated nutraceutical and cosmetic products.[7]

EXPERIMENTAL

Materials and reagents

HPLC-grade methanol and isopropanol (IPA) were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Denatured ethanol (reagent grade) was purchased from Sigma (St. Louis, MO, USA). The fine ground plant material was used as received.

Sample preparation

Solvent extraction

A total of 0.3 g of ground plant material and 6 mL methanol were placed into a 10 mL test tube. After sonication at 40 °C for 1 hour, the suspension was centrifuged for 5 min. The supernatant was transferred to a clean vial for further analysis.

Supercritical fluid extraction

The extraction experiments were performed on a Waters® MV-10 ASFE® System controlled by ChromScope Sample Prep Software. A total of 3 g of ground plant material was weighed into a 5 mL extraction vessel. The extraction was performed for 60 minutes with 8 mL/min CO_2. The effluent was carried into a 100 mL collection vessel with a makeup flow of 1 min/mL of methanol/isopropanol/hexane (1:1:1).

Chromatography

Analytical LC-MS experiments were performed on a Waters ACQUITY UPLC H-Class System/SQ Detector 2 and a Waters AutoPurification LC System. The analytical UPC^2-MS experiments were performed on a Waters ACQUITY UPC^2-MS System. All systems were controlled by MassLynx software. The MS-directed SFC preparative experiments were performed on a Waters Prep 100q SFC MS-Directed System controlled by MassLynx/FractionLynx Software. All UV-directed preparative experiments were performed on a Waters SFC 80 Preparative System controlled by ChromScope software. Detailed experimental parameters are summarized in Tables 1-3.

To that end, supercritical fluid (SF) based techniques, including supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC), can offer viable additions to the natural product isolation toolbox by leveraging the unique properties of supercritical CO_2: high diffusivity, low viscosity, and superb solvation power. SFE has been successfully applied to the extraction of many bioactive compounds from medicinal plants, including steroids, terpenes, alkaloids, and phenolic compounds.[6] Preparative SFC has been widely adopted by the pharmaceutical industry for active pharmaceutical ingredient (API) purification. Its applications in natural product isolation, however, remain scarce.[8]

In this application note, we describe a systematic effort to holistically improve the productivity in isolating a naturally occurring terpene derivative with proven anti-cancer bioactivity from a raw plant sample. The process involves an extraction by SFE followed by three different, two-step purification routes, including MPLC+HPLC, MPLC+SFC, and SFC+SFC. The overall productivity and solvent consumption for each purification route are compared.

	Figure 2A		Figure 2B		Figure 6A	
Instrument	ACQUITY UPLC H-Class System/SQD2 MS		AutoPurification LC MS System		ACQUITY UPLC H-Class System/SQD2 MS	
Flow rate (mL/min)	0.60		1.46		0.75	
Mobile phase A	Water		Water		Water	
Mobile phase B	Methanol		Methanol		Methanol	
Backpressure (psi)	N/A		N/A		N/A	
MS detection	ESI+		ESI+		ESI+	
Column	ACQUITY HSS T3 (1.8 µm, 3.0 x 150 mm)		Atlantis T3 (5 µm, 4.6 x 150 mm)		ACQUITY BEH C_{18} (1.7 µm, 2.1 x 50 mm)	
Temperature (°C)	60		Ambient		60	
Injection volume (µL)	1		Varying		0.5	
Gradient	Time (min)	%B	Time (min)	%B	Time (min)	%B
	0	92	0	88	0	80
	5	96	3.08	88	4	80
	5.25	92	8.21	94		
	6	92	8.61	100		
			9.22	88		
			20.90	88		

Table 1. Key experimental parameters for analytical LC.

	Figure 1		Figure 3A		Figure 4A		Figure 4B		Figure 6B	
Instrument	ACQUITY UPC² System/TQD MS									
Flow rate (mL/min)	1.5									
Backpressure (psi)	1740									
MS Detection	APCI+									
Temperature (°C)	45									
Injection volume (µL)	1									
Mobile phase A	CO_2									
Mobile phase B	Methanol		Isopropanol		Isopropanol		Isopropanol		Isopropanol	
Column	ACQUITY UPC² 2-EP (1.7 µm, 3.0 x 100 mm)		ACQUITY UPC² 2-EP (1.7 µm, 3.0 x 100 mm)		ACQUITY UPC² 2-EP (1.7 µm, 3.0 x 100 mm)		GreenSep Nitro (1.8 µm, 3.0 x 100 mm)		ACQUITY UPC² 2-EP (1.7 µm, 3.0 x 100 mm)	
Gradient	Time (min)	%B	Time (min)	%B	Time (min)	%B	Time (min)	%B	Time (min)	%B
	0	5	0	5	0	5	0	20	0	5
	4.5	45	2.50	25	2.50	25	2.5	50	2	25
	5	45	2.75	40	2.75	40	3	50	2.75	40
	5.25	5	3.25	40	3.25	40	3.25	20	3.25	40
	6	5	3.50	5	3.50	5	4	20	3.50	5
			4	5	4	5			4	5

Table 2. Key experimental parameters for UPC.²

	Figure 3B		Figure 5A		Figure 5B	
Instrument	Prep 100q SFC MS-Directed System		SFC 80 Preparative System		SFC 80 Preparative System	
Flow rate (mL/min)	80		80		80	
Mobile phase A	CO_2		CO_2		CO_2	
Mobile phase B	Isopropanol		Isopropanol		Ethanol	
Backpressure (psi)	1740		1740		1740	
Column	Viridis Silica 2-EP (5 µm, 19 x 150 mm)		Viridis Silica 2-EP (5 µm, 19 x 150 mm)		Nitro (5 µm, 21 x 150 mm)	
Temperature (°C)	40		40		40	
Sample diluent	Isopropanol		Isopropanol		Ethanol	
Injection volume (mL)	0.6		3		1	
Collection trigger	MS		UV		UV	
Gradient	Time (min)	%B	Time (min)	%B	Time (min)	%B
	0	5	0	5	0	8
	1	5	5	5	9	8
	6.5	9	7	30		
	7	9	10	30		
	7.25	5	11	5		
	8	5	12	5		

Table 3. Key experimental parameters for preparative chromatography.

RESULTS AND DISCUSSION

Target compound extraction using SFE

Any solid-liquid extraction process, such as solvent extraction and SFE, is predominantly a solubility driven process. The process involves diffusion of the extracting solvent into the matrix, solubilization of the target analytes in the extracting solvent, diffusion of the target analytes in the extraction solvent, and transport of the extracted analytes into a collection vessel.[6] Conventional polar extraction solvents, such as alcohols, often produce extracts comprised of mixtures of many polar and non-polar compounds. Supercritical CO_2, on the other hand, is a highly lipophilic solvent. As a result, only relatively non-polar compounds are typically extracted by SFE using neat CO_2. In the current study, the target compound is a terpene derivative with a nominal mass of 390.28 Da and a LogP of 3.0. The low molecular weight and the relatively low polarity make it an ideal candidate for extraction by SFE.

Figure 1 shows the UPC^2-MS chromatograms of two extracts obtained by SFE (Figure 1A) and methanol extraction (Figure 1B) using a BEH 2-EP column. Since 2-EP is a polar stationary phase, the elution order of the compounds generally tracks their polarities; the later the elution, the more polar the compounds. While both extracts contain similar amount of the target compound, it is evident that SFE yielded a much simpler extract compared to methanol extraction. For the SFE extract, the peaks immediately after the target compound (1.20–1.75 min, blue rectangle) are much lower in intensity than those in the methanol extract. The peaks between 1.75–3.50 min (red rectangle) are only present in the methanol extract. Overall, the SFE extract is a much simpler mixture consisting of fewer polar components. The target compound was therefore enriched by SFE prior to chromatography. This makes the SFE extract ideal for large mass loading in prep chromatography and requires relatively low organic co-solvent (mobile phase B) composition to completely elute off the components in the extract; thereby shortening the total run time, reducing the solvent consumption, and increasing purification productivity. Detailed prep SFC experiments are described in a later section.

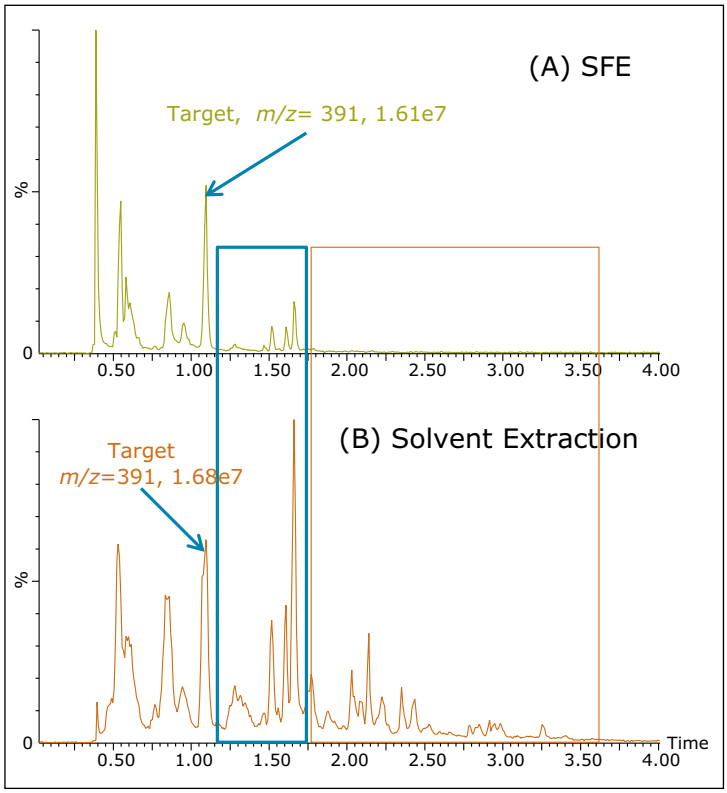

Figure 1. UPC^2-MS chromatograms of the mixtures obtained by (A) SFE and (B) methanol extraction, using an ACQUITY UPC^2 2-EP Column.

Conventional purification approach: MPLC + HPLC

One of most commonly used approaches in natural product isolation involves MPLC followed by HPLC. In the current case, the SFE extract first underwent a purification step by MPLC (results not shown), attaining the target compound of >97% purity (referred to as the MPLC fraction hereafter). The main remaining impurity has a nominal mass of 360.27 Da, and results from the demethoxylation of the target compound. The structural similarity between the target and impurity presented a challenge in RPLC purification. Figure 2A shows the UPLC®-MS and UV chromatograms of the MPLC fraction. A baseline resolution between the target and the impurity was achieved using a 3.0 x 150 mm UPLC column, where the impurity was present as a sodium adduct with an *m/z*=383. The close elution of the two peaks, however, severely hampered the sample loadability in the ensuing RPLC purification. Figure 2B summarizes a loading study of the MPLC fraction on an analytical column (5 µm, 4.6 x 150 mm). The baseline resolution was only preserved with a 10-µL injection. With an 80-µL injection, the impurity peak completely merged into the target peak. In addition to the limited resolution, the elution order of the compounds also contributed to the low purification productivity. With RPLC, the impurity elutes before the target compound. In the case where target and impurity are partially separated, such as the one with 40-µL injection in Figure 2B, though it is still possible to obtain pure target compound by excluding the front of the target peak where the impurity co-elutes, such practice is generally inadvisable in prep chromatography as the front of a peak often accounts for a high percentage of the total peak. Based on the loading study performed on the analytical column, the maximum loading on a 19 x 150 mm semi-prep column without compromising yield or purity was projected to be 170 µL. At ~20 mg/mL, this translates into a maximum loading of 3.4 mg/injection.

Leveraging the orthogonality between RPLC and SFC for improved loading capacity: an MPLC + SFC approach

SFC offers an attractive alternative. SFC is generally considered a normal-phase chromatographic technique when a polar stationary phase, such as 2-EP, is used. As a result, the elution order often reverses that in RPLC using a non-polar C_{18} column. Figure 3A shows the UPC^2-MS and UV chromatograms of the MPLC fraction using a BEH 2-EP column. Compared to Figure 2A, not only did the UPC^2 method provide a better resolution, the elution order of the target and the impurity also reversed. The chromatography was then scaled up to a 19 x 100 mm semi-prep column, and the resulting chromatogram is shown in Figure 3B. The resolution was well maintained with a 600-µL injection at 20 mg/mL. The total run time using SFC was 8 min compared to the 20-min run time using RPLC. By using prep SFC to replace prep RPLC, the overall productivity was increased by 9-fold: 2.5-fold from the reduced run time and 3.5-fold from the increased sample loading.

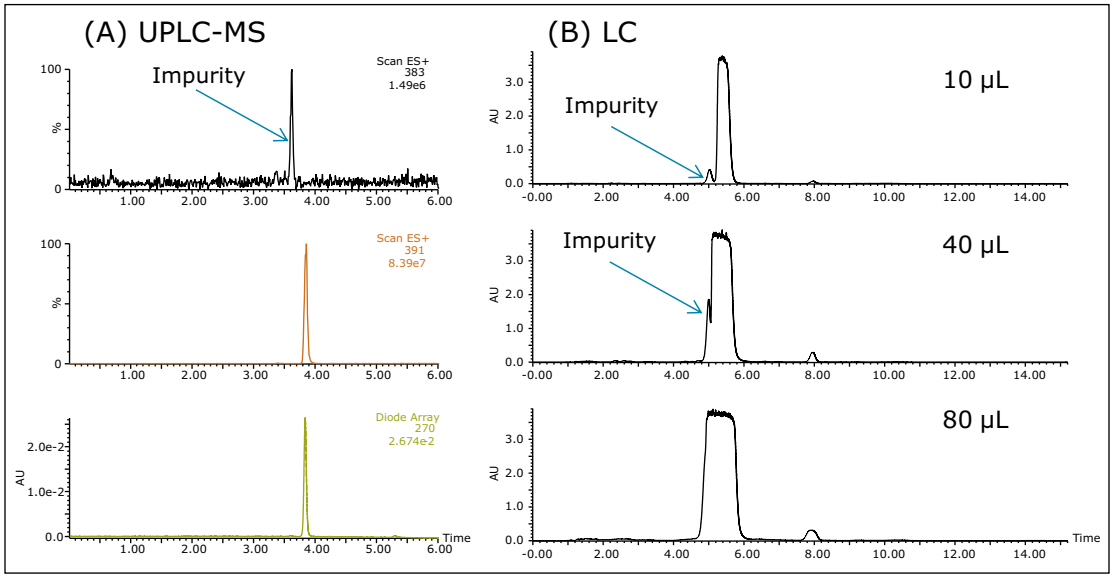

Figure 2. (A) UPLC-MS and UV chromatograms of the MPLC fraction at 1 mg/mL; and (B) LC/UV chromatograms of the MPLC fraction at 20 mg/mL.

Leveraging the orthogonality between different column chemistries in SFC for improved purification productivity: an SFC + SFC approach

Though the approach demonstrated in Figure 3 led to a notable improvement in productivity, the overall process still suffers from large solvent consumption, mainly due to the initial MPLC step. The target compound in the current study has a relatively low polarity. For this sample, a high percentage of organic solvent is required to elute the target compound in LC; hence, the large solvent consumption. In SFC, however, the lipophilic CO_2 is the main mobile phase that elutes the target compound, thus minimizing the use of organic solvents (mobile phase B). Moreover, the raw sample was extracted with neat CO_2 and is, therefore, inherently compatible with SFC.

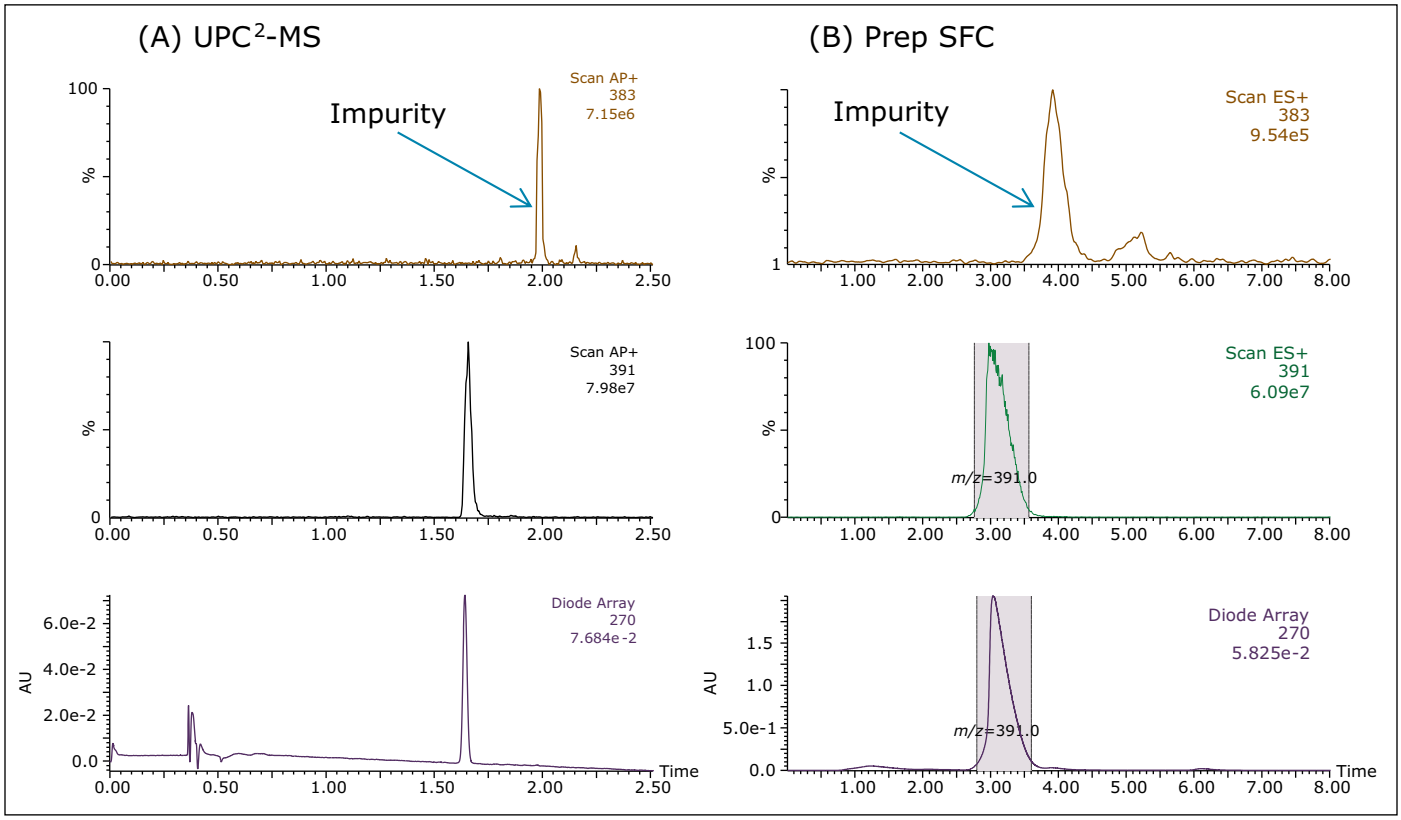

Figure 3. (A) UPC²-MS and UV chromatograms of the MPLC fraction at 1 mg/mL; and (B) prep SFC-MS and UV chromatogram of the MPLC fraction at 20 mg/mL.

There is a wide range of column chemistries available in SFC, with retention mechanisms encompassing polar interactions, hydrophobic interactions, π-π interactions, and steric recognitions. With proper selection of column chemistries, SFC can offer orthogonal selectivity necessitated by the sample complexity intrinsic to natural product isolation. Figure 4 shows the UPC²-MS and UV chromatograms of the SFE extract using a BEH 2-EP (Figure 4A) and a nitro column (Figure 4B), respectively. While 2-EP columns typically render polar interactions between analytes and stationary phase, nitro columns often retain and separate analytes based on π-π interactions. This kind of combination provides complementary separation around the target compound. As can be seen in Figure 4, using a 2-EP column, the target compound at *m/z*=391 is well separated from the impurity at *m/z*=361, but less separated from another later eluting impurity at *m/z*=239. In contrast, using a nitro column, the impurity at *m/z*=239 became an earlier eluting peak and was well separated from the target compound, but the impurity at *m/z*=361 co-eluted with the target compound.

Leveraging the orthogonality between different column chemistries in SFC for improved purification productivity: an SFC + SFC approach

Though the approach demonstrated in Figure 3 led to a notable improvement in productivity, the overall process still suffers from large solvent consumption, mainly due to the initial MPLC step. The target compound in the current study has a relatively low polarity. For this sample, a high percentage of organic solvent is required to elute the target compound in LC; hence, the large solvent consumption. In SFC, however, the lipophilic CO_2 is the main mobile phase that elutes the target compound, thus minimizing the use of organic solvents (mobile phase B). Moreover, the raw sample was extracted with neat CO_2 and is, therefore, inherently compatible with SFC.

Based on the retention behavior illustrated in Figure 4, a two-step SFC purification strategy was implemented: using a 2-EP column to remove the main impurity with an *m/z*=361 followed by using a nitro column to remove any remaining impurities after the first step, such as the one with an *m/z*=239. The resulting chromatograms are shown in Figure 5. The overall yield, defined as the weight of the purified pure target compound/the total weight of SFE extract taken for purification, was similar to those from the other two approaches: MPLC+HPLC and MPLC+SFC.

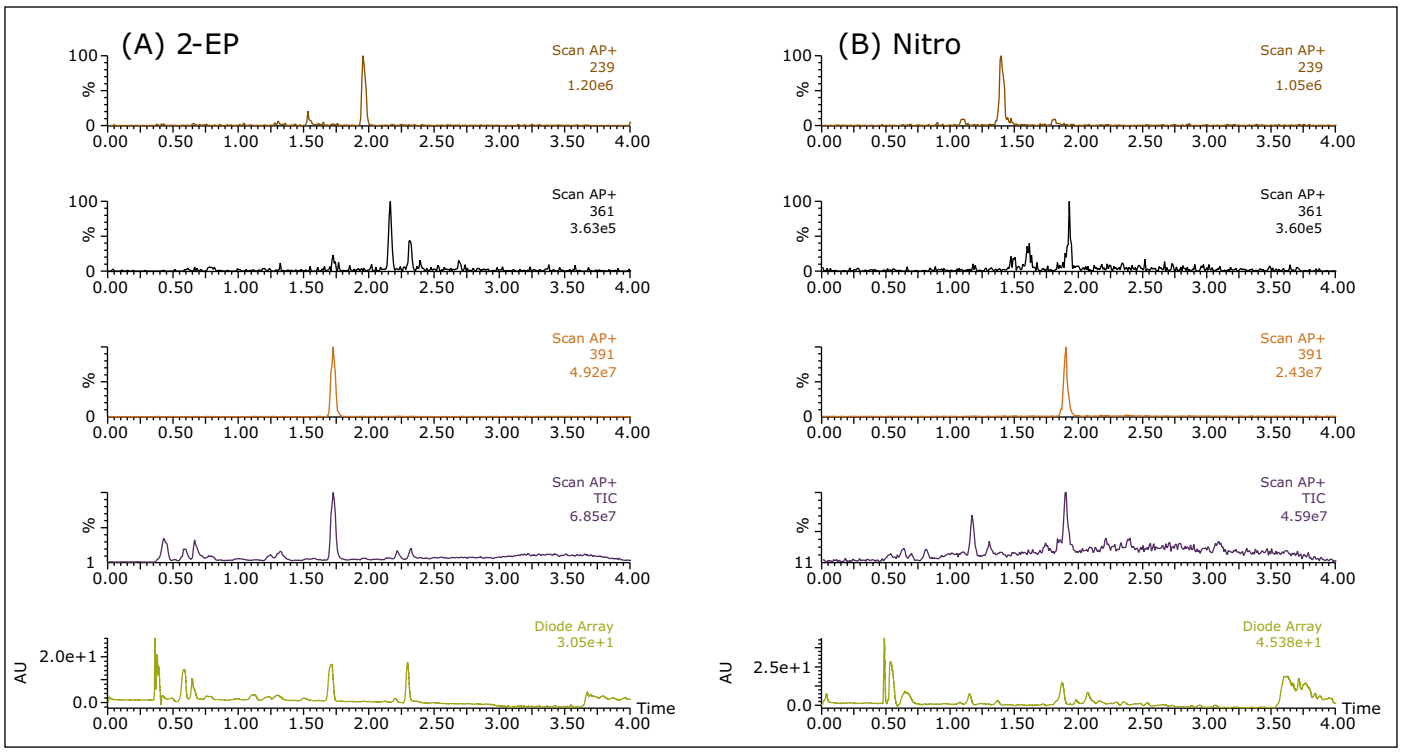

Figure 4. UPC²-MS and UV chromatograms of the SFE extract using (A) a 2-EP; and (B) a nitro column.

Aliquots of the purified final product were analyzed by both UPC²-MS and UPLC-MS to ensure a true representation of the sample profile. The resulting chromatograms are shown Figure 6. Both impurities at *m/z*=361 and *m/z*=239 illustrated in Figure 4 were successfully removed. The results indicate that the final product has a purity >99% by UV.

The SFC purification process resulted in smaller fraction volumes compared to MPLC and HPLC. The SFC fractions were quickly dried under mild conditions, minimizing the possible compound loss due to thermal degradation associated with the post-purification dry-down process. Compared to LC, SFC offered an easier
and faster compound recovery.

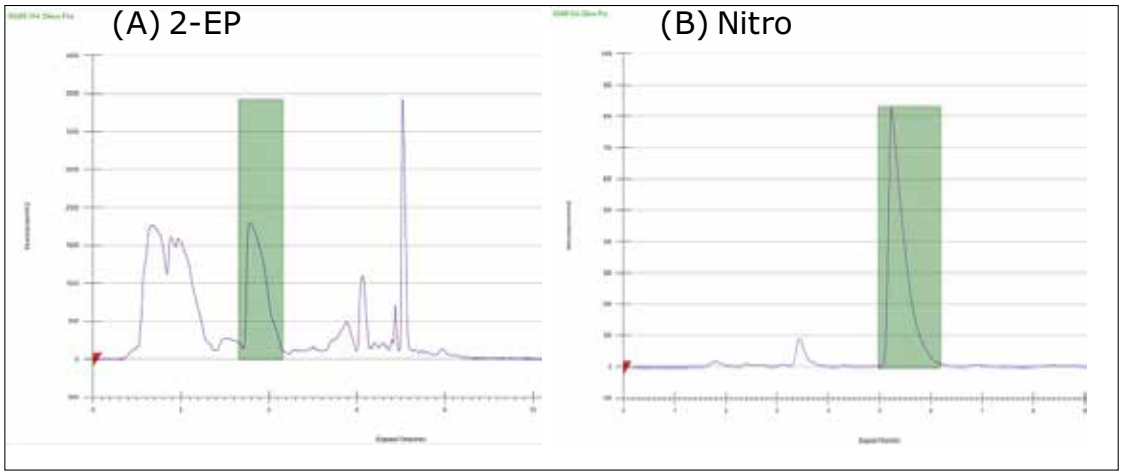

Figure 5. (A) SFC/UV chromatogram of the SFE extract at 133 mg/mL using a Viridis 2-EP column; and (B) SFC/UV chromatogram of the collected fraction from the Viridis 2-EP step on a nitro column.

The SFC purification process resulted in smaller fraction volumes compared to MPLC and HPLC. The SFC fractions were quickly dried under mild conditions, minimizing the possible compound loss due to thermal degradation associated with the post-purification dry-down process. Compared to LC, SFC offered an easier and faster compound recovery.

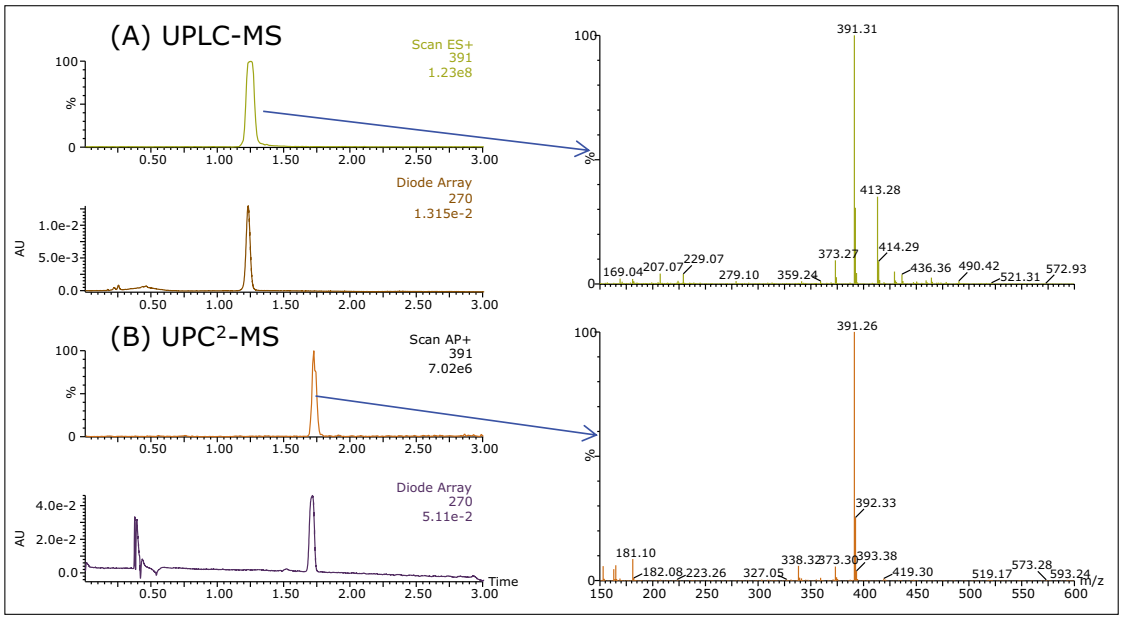

Figure 6. Purity analysis of the final product by (A) UPLC-MS and (B) UPC^2-MS.

Process	Rate-limiting step	Productivity (g/24 hr)	Solvent	Solvent consumption (L/24 hr)	CO_2 use (kg/24hr)
MPLC+ HPLC*	HPLC	0.25	MeOH	95	N/A
MPLC+SFC**	SFC	2.25	MeOH/IPA	75	105
SFC+SFC	First step SFC	3.50	IPA/Ethanol	11	105

**HPLC calculations were based on a 19 x 150 mm column.*

***SFC calculations were based on 19 x 150 mm columns.*

Table 4. Comparison on productivity and solvent consumption of different purification processes.

CONCLUSIONS

In this application note, we have demonstrated employing SFE and prep SFC to holistically improve the productivity in isolating a low-polarity, bioactive compound from a complex natural product extract. The SFE alleviated the sample complexity prior to analysis and purification, thereby improving sample loading and reducing solvent use in the ensuing chromatography. The SFE extract also lends itself well for SFC analysis and purification.

For the MPLC+HPLC purification route, the target compound and its demethoxylated derivative formed a critical pair in HPLC that limited the column loading and overall purification productivity. The same critical pair was better separated on a 2-EP column using SFC. The elution order of the pair was also altered, enabling an increased column loading. Overall, the MPLC+SFC route offered a 9-fold improvement in productivity. However, both routes still suffered from large solvent consumption because of the MPLC step. Finally, an SFC+SFC purification process was developed, leveraging the orthogonal selectivity between different column chemistries available in SFC. The SFC+SFC route not only led to a 16-fold improvement in productivity, but also a 90% reduction in solvent consumption. In addition, both SFE and SFC also provided an easy sample recovery under mild conditions that minimized potential compound loss due to thermal degradation associated with post-purification dry-down.

The supercritical fluid-based techniques, SFE and SFC, augment the conventional toolbox for natural product research by offering unique selectivity in both extraction and chromatography; and empower laboratories and manufacturers in pharmaceutical, traditional medicine, nutraceutical, dietary supplement, and cosmetic industries for more efficient and more cost-effective natural product isolation and purification.

References

1. Harvey AL, Strategies for discovering drugs from previously unexplored natural products. *Drug Discovery Today*, 2000; 5 (7):294–300.
2. Harvey AL, Natural products in drug discovery. *Drug Discovery Today*, 2008; 13 (19/20):894–901.
3. Li JWH, Vederas JC, Drug Discovery and natural products: end of an era or endless frontier? *Science*, 2009; 325 (10):161–165.
4. Harvey AL. Natural Products as a screening source, *Curr. Opin. Chem. Biol.*, 2007, 11: 480–484.
5. Sarker SD, Latif Z, Gary AI, Natural product isolation: an overview, *Natural Product Isolation, 2nd ed*. Eds. Sarker SD, Latif Z, Gary AI, Humana Press Inc. Totowa, NJ. 2006, P1–25.
6. Sticher O, Natural product isolation, *Nat. Prod. Rep.*, 2008, 25, 517–554.
7. Koehn FE, Carter G, The evolving role of natural products in drug discovery, *Nat. Rev. Drug Discov.* 2005, 4: 206–220.
8. Ramirez P, Garcia-Risco MR, Santoyo S, Senorans FJ, Ibanez E, Reglero G, *J. Pharmaceu. Biomed.* 2006, 41:1606–1613.

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[TECHNOLOGY BRIEF]

Rapid Analysis of Complex Heterogeneous Mixtures for Active Components in Cosmetic Products

GOAL

To employ Waters® Atmospheric Solids Analysis Probe (ASAP) as a sample inlet for mass spectrometry to directly analyze heterogeneous samples and eliminate sample preparation.

Streamline your workflow when monitoring mixtures in targeted analyses with ASAP, which provides mass spectra of mixtures within seconds – no sample workup required.

BACKGROUND

Active components, such as UV absorbers are widely used in formulated personal care products, including sun block and over-the-counter facial creams. These components are constantly under examination for quality control, thermal stability or shelf life, and photochemical stability. The sample matrix is a heterogeneous complex mixture that includes components as diverse as pigments, oils, emulsions, and functional or active organic chemicals. Typical analyses of these types of materials include the use of conventional analytical tools, such as NMR, LC, or LC-MS. These techniques require time-consuming workup procedures that include precipitation, extraction, filtration, separation, and evaporation. ASAP can provide mass spectra of mixtures within seconds without sample workup, which streamlines the workflow when monitoring mixtures in targeted analysis.

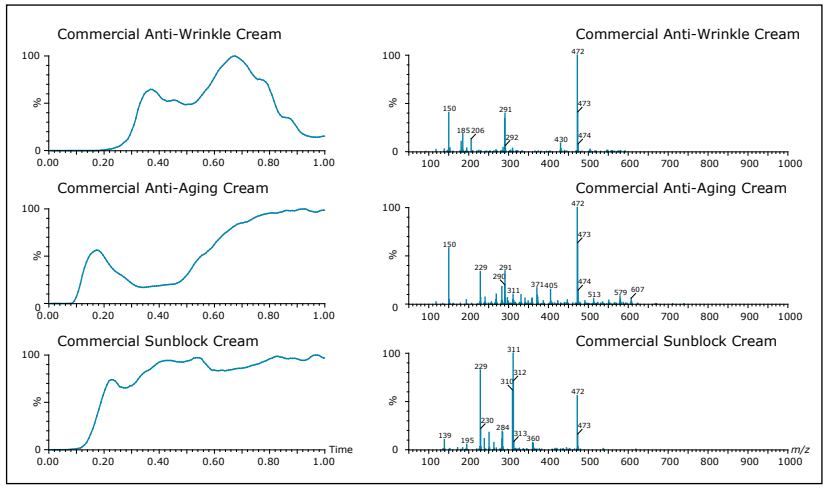

Figure 1. The complex sample spectra were recorded for the bulk sample without dilution, extraction, or any sample pre-treatment. Note that both negative and positive ionization were collected – positive ionization data are displayed.

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THE SOLUTION

A tandem quadrupole mass spectrometer with an ASAP probe was used to analyze critical components in formulated products, without the need for extensive sample preparation or isolation of the analyte from a heterogeneous matrix. The sample was loaded onto a glass tube on the probe by dipping its tip directly into the product mixture. The probe was inserted into the MS source at atmospheric pressure. Desolvation gas heated to 350 °C was used to volatilize the analytes. Mass spectra were acquired in two minutes using both APCi positive and negative mass scan modes. The targeted analytes were isolated from matrix interference based on confirmatory fragmentation.

A complex data set is displayed in the collected spectra, shown in Figure 1, which provide a summation of information from a wide array of product components. Utilizing a targeted workflow approach, an evaluation of the data set focused on expected and other typical components in the various products.

Employing a wide array of collision conditions for the target analyte list, the sample components were analyzed to determine appropriate fragmentation conditions.

Based on the collision cell conditions for each component in the target analyte list, a schedule of MRM scans was established. The samples were then reanalyzed using the ASAP sampling method, and the resulting thermal desorption chromatograms were collected, as shown in Figure 2.

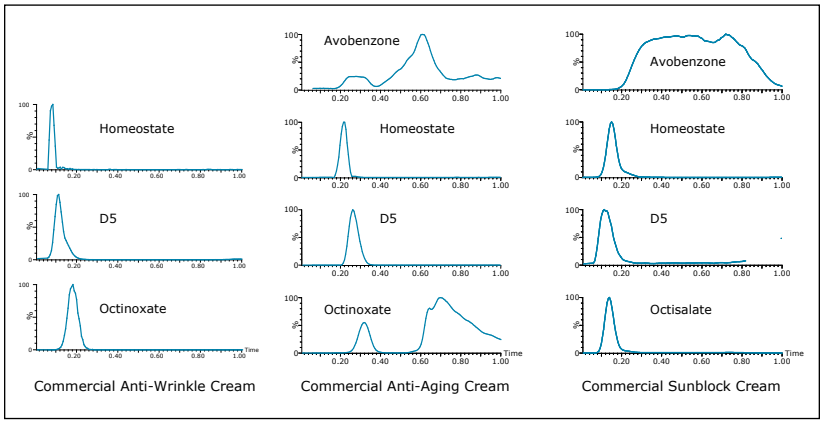

Figure 2. Thermal desorption chromatograms for the target analyte list using MS/MS data. Each thermal chromatogram was processed using a simple smoothing function to provide consistent data over the acquisition range.

Analysis of active ingredients in personal care products was routinely conducted using ASAP as a mass spectrometer inlet with a total analysis time of two minutes. This technique allowed for direct sample introduction, without the need for sample preparation. Complex sample matrix interferences were easily addressed with a targeted analysis using established fragmentation patterns produced in the collision cell, which resulted in unique analyte detection.

SUMMARY

- Using ASAP as a sample inlet for analysis of heterogeneous sample matrices allows for collection of characteristic mass spectra and analysis of relative concentration of components in a product mixture.
- This analytical approach can be used to monitor key ingredients, as well as to profile product integrity.
- ASAP can provide critical data and increased analysis capacity with minimal specialized operator training required to support researchers, as well as production operations.

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Enantiomeric and Diastereomeric Separations of Fragrance and Essential Oil Components using the ACQUITY UPC² System with ACQUITY UPC² Trefoil Columns

John P. McCauley and Rui Chen
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Shorter analysis times compared to chiral GC.
- The 2.5-µm particle chiral stationary phases provide high efficiency enantiomeric separations for fragrance compounds.
- The low system volume and extra-column volume of the ACQUITY UPC² System enables superior, faster, and more efficient enantiomeric separations of fragrance compounds compared to traditional SFC.
- UPC² solvents are more compatible with mass spectrometry, compared to those used in normal-phase chiral HPLC, enabling superior real time peak identification.

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ACQUITY UPC²® Trefoil™ AMY1 and CEL1 2.5 µm Columns

ACQUITY UPC² System with ACQUITY UPC² PDA Detector and ACQUITY® TQ Detector

MassLynx® Software

KEY WORDS

Enantiomers, chiral stationary phase, fragrance, essential oils, UltraPerformance Convergence Chromatography (UPC²), convergence chromatography (CC), Trefoil

INTRODUCTION

Perception of aroma occurs at the olfactory membrane. This membrane is comprised in part of proteins and carbohydrates, which are chiral in nature. This makes it possible for the olfactory receptors to distinguish between enantiomers. Many enantiomers of fragrance molecules are perceived differently by our sense of smell.[1] For example, carvone is a chiral terpenoid where the R enantiomer smells like spearmint while the S enantiomer has the distinct odor of caraway seed.[2]

Chiral composition of fragrance molecules is important for many industries, including food, cosmetics, and consumer products, in controlling the olfactory perception of products.[1] In addition, chiral analyses are routinely performed to authenticate the natural sources of essential oils. Since naturally chiral sources of essential oils are generally more costly and have a greater perceived health benefit than their synthetic counterparts, rapid chiral analysis allows manufacturers to quickly exclude adulterated products containing inexpensive racemic synthetic materials at the time of purchase.[3]

Historically, chiral separations of fragrance compounds have primarily been carried out using chiral stationary phases (CSPs) in capillary gas chromatography (GC).[2,3,4] The analysis time often ranges from 15 to 50 minutes.[3] More recently, supercritical fluid chromatography (SFC) with CSPs has been applied to these separations, often resulting in comparable resolution and reduced run time.[5,6] Despite the great success in chiral separation by SFC, the associated instrumentation and CSPs have been slow to tap into the technology advancements that have taken place in the HPLC field. For example, one of most significant breakthroughs in HPLC in the past decade is the advent of Waters® UPLC® Technology, which utilizes sub-2-µm particles. ACQUITY UPLC® Systems retain the practicality and principles of HPLC while increasing the overall interlaced attributes of speed, sensitivity, and resolution. Until very recently, the standard particle size for commercially available CSPs has remained 5 µm.

Convergence chromatography is the next evolution in SFC. The Waters ACQUITY UPC² System is a holistically designed system that has similar selectivity to normal-phase chromatography and is built upon proven UPLC Technology.

EXPERIMENTAL

Instrumentation

All experiments were performed on an ACQUITY UPC^2 System equipped with an ACQUITY UPC^2 PDA Detector and an ACQUITY TQ Detector. The system is controlled by MassLynx Software.

Samples

The standard samples used in this study were purchased from TCI Americas, with their structures shown in Figure 1. Essential oils were purchased from various commercial sources. All samples were dissolved in tert-butyl methyl ether (TBME) for the analyses.

UPC^2 conditions

Column: ACQUITY UPC^2 Trefoil AMY1 or CEL1 (2.5 µm, 3.0 x 150 mm)

Backpressure: 1740 psi

Temperature: 40 °C

Mobile phase A: CO_2

Mobile phase B: Isopropanol.

MS: APCI positive mode.

Other key parameters are listed in their respective figure captions.

UltraPerformance Convergence Chromatography™ (UPC$^{2®}$) offers minimized system and dwell volume, enabling users to leverage the superior separation power inherent to smaller particle sizes.

We present herein the enantiomeric and diastereomeric separations of four fragrance compounds using Waters ACQUITY UPC^2 Trefoil AMY1 and CEL1 Columns on an ACQUITY UPC^2 System. Compared to the traditional method of analysis by GC, UPC^2 offered similarly high resolution with significantly shorter run times, resulting in improved productivity.

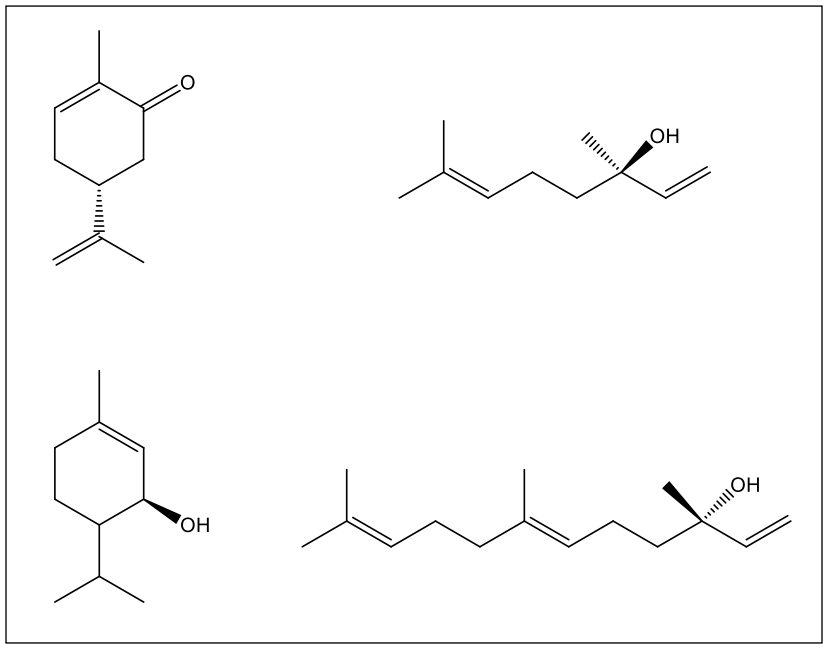

Figure 1. Structures of the four fragrance compounds presented in this study.

RESULTS AND DISCUSSION

Figure 2 shows the UPC^2-UV chromatogram of carvone enantiomers on an ACQUITY UPC^2 Trefoil CEL1 Column. The enantiomeric pair was baseline resolved in less than 2.5 min (Figure 2C). The peak widths at half-height are 2-3 s. It is also interesting to note that there are detectable antipodes present in both single enantiomer standards (Figures 2A and 2B). In both cases, the minor peaks account for approximately 1% of the main peaks, resulting in a 98% enantiomeric excess (e. e.). This example clearly demonstrates a high efficiency chiral separation enabled by a 2.5-µm CSP on an ACQUITY UPC^2 System, resulting in short analysis time, sharp peaks, and improved detection sensitivity.

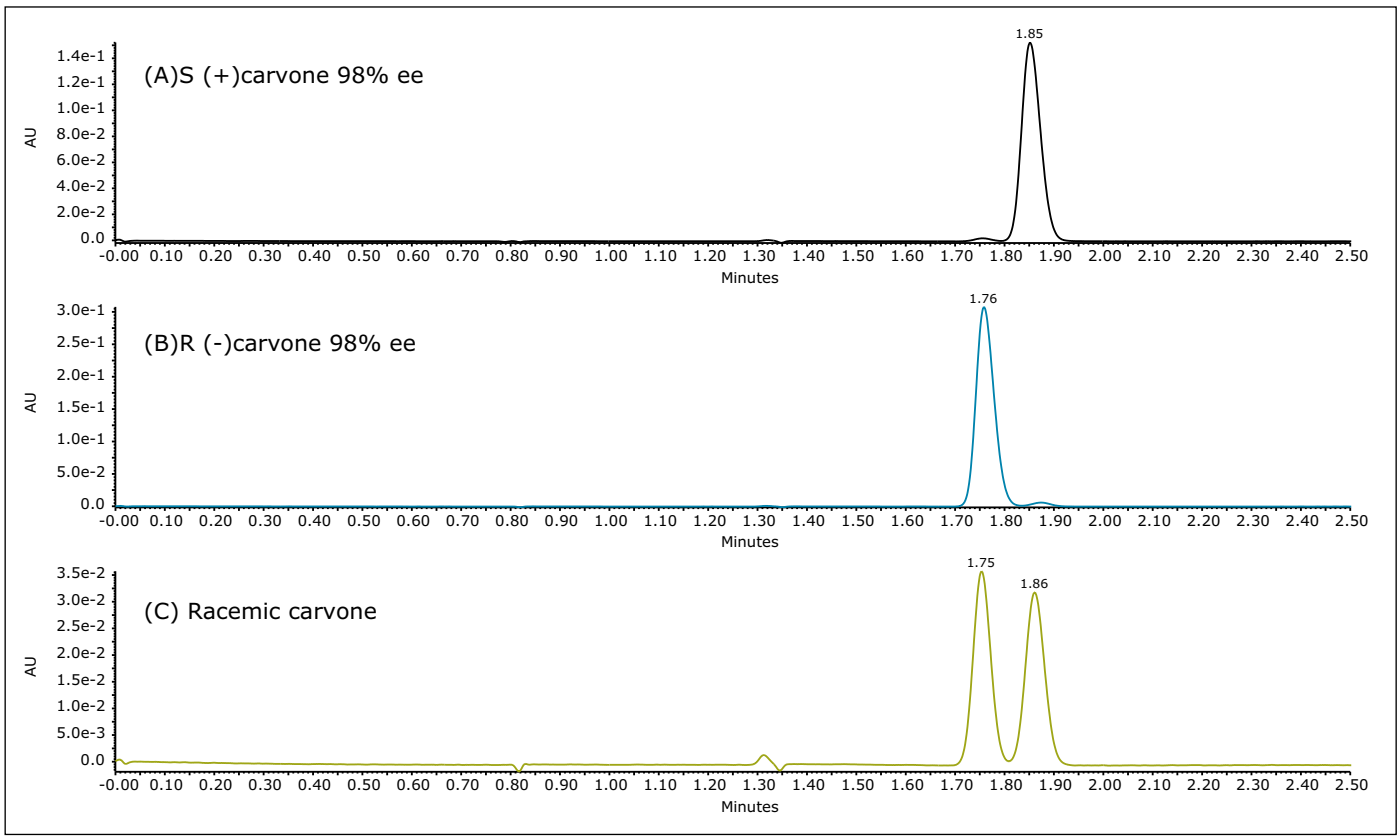

Figure 2. UPC^2-UV chromatograms of the enantiomeric separation of carvone on an ACQUITY UPC^2 Trefoil CEL1 Column: (A) S (+) carvone; (B) R (-) carvone; and (C) racemic carvone. An isocratic method with 4% isopropanol was used. The flow rate was 0.9 mL/min.

Linalool is a terpene alcohol with a soft floral odor, and can be found in different plant extracts. Figure 3A shows the enantiomeric resolution of the linalool standard on an ACQUITY UPC^2 Trefoil AMY1 Column. It is noted that the linalool standard was non-racemic (Figure 3A), suggesting the standard was derived from a natural source. The e. e. was estimated to be 40% in favor of the late eluting isomer. Figure 3B is the UPC^2-UV chromatogram of a commercially available lavender essential oil obtained under the same condition. The two linalool enantiomers were identified by both retention time and corresponding mass spectra (results not shown). It is noted that MS plays a critical role for the positive identification of the target analytes in a complex matrix. While bearing a similar selectivity to normal-phase LC, UPC^2 is inherently advantageous in incorporating MS detection due to its MS-friendly mobile phase. The linalool in this lavender essential oil exhibited a 92% e. e. in favor of the later eluting peak at 2.07 min.

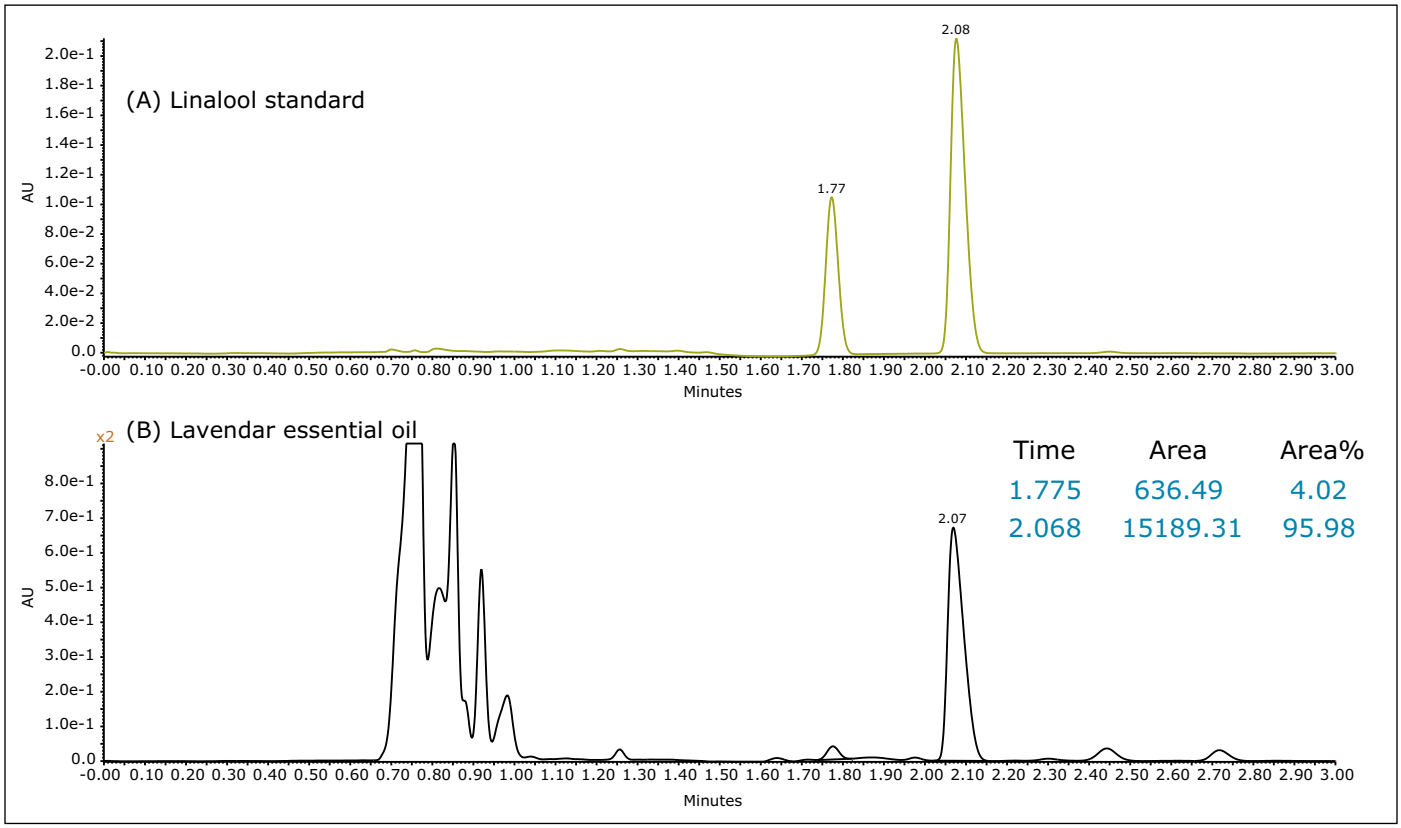

Figure 3. UPC^2-UV chromatograms of (A) linalool standard (B) lavender essential oil on an ACQUITY UPC^2 Trefoil AMY1 Column. An isocratic method with 3% isopropanol was used for linalool. The flow rate was 1.0 mL/min.

Similarly, terpinen-4-ol is a terpene with a pleasant conifer odor, and is a major constituent of tea tree oil. Figure 4A shows the enantiomeric resolution of the two isomers of a terpinen-4-ol standard on an ACQUITY UPC^2 Trefoil™ AMY1 Column. The terpinen-4-ol standard was nearly racemic (Figure 4A), suggesting its synthetic origin. Examination of a tea tree essential oil (Figure 4B) revealed that the terpinen-4-ol exhibited a 37% e. e. in favor of the early eluting isomer at 1.95 min.

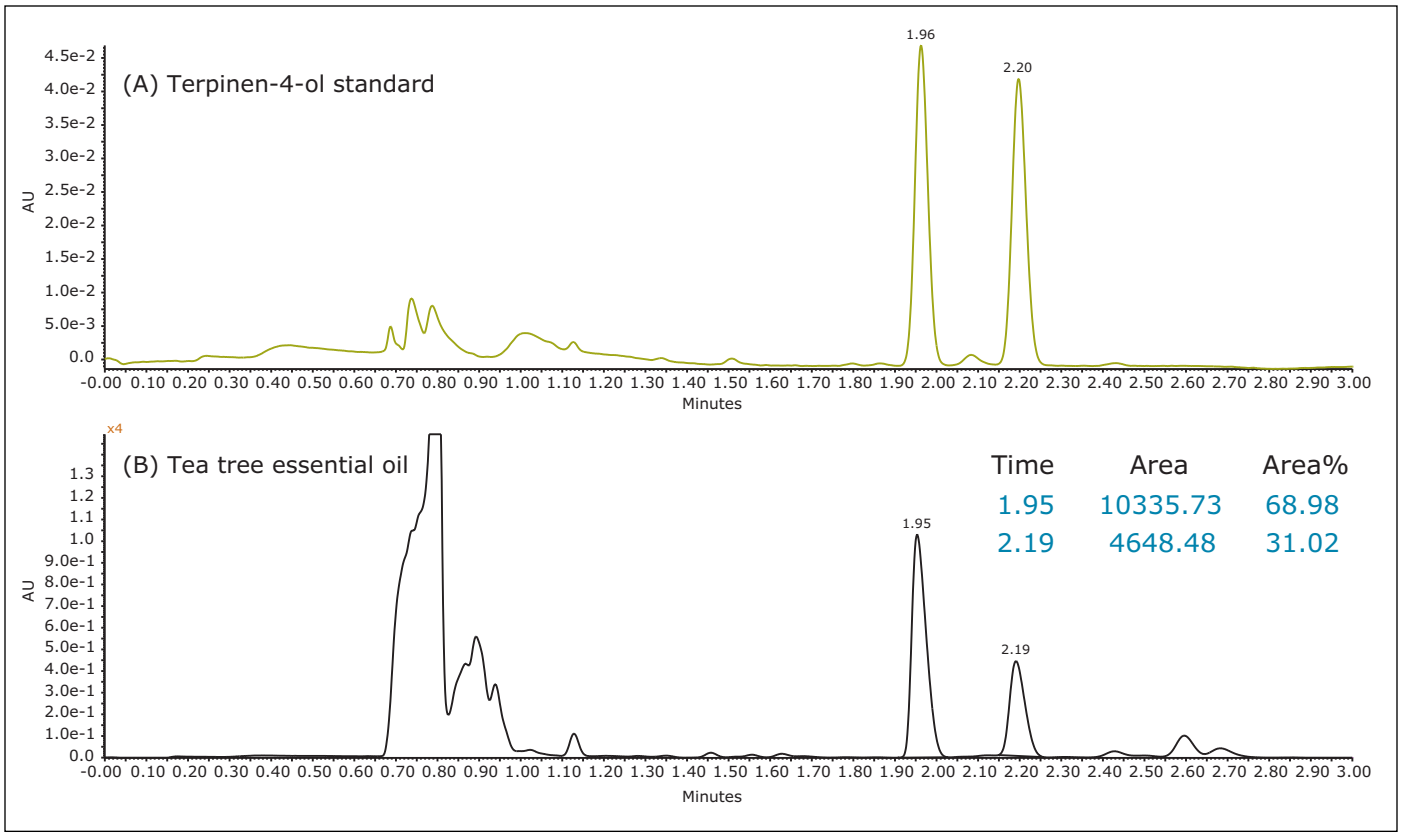

Figure 4. UPC^2-UV chromatograms of (A) Terpinen-4-ol standard and (B) Tea Tree essential oil on an ACQUITY UPC^2 Trefoil AMY1 column. An isocratic method with 5% isopropanol was used. The flow rate was 1.0 mL/min.

Nerolidol, which can be found in the neroli essential oil derived from the bitter orange plant, is a sesquiterpene with a pleasant woody odor reminiscent of fresh bark. The nerolidol molecule (Figure 1) contains a chiral center and a double bond generating cis/trans isomerism, resulting in four possible stereoisomers in a mixture. Figure 5 shows the simultaneous separation of all four nerolidol isomers on an ACQUITY UPC^2 Trefoil AMY1 column in less than 3 min. Figure 5B is the selected ion recording (SIR) for the isomeric mixture at *m/z* 205.2, corresponding to the $[(M+H)-H_2O]^+$ of nerolidol. The observation of the base peak of nerolidol resulting from the loss of water is consistent with other reports.[7]

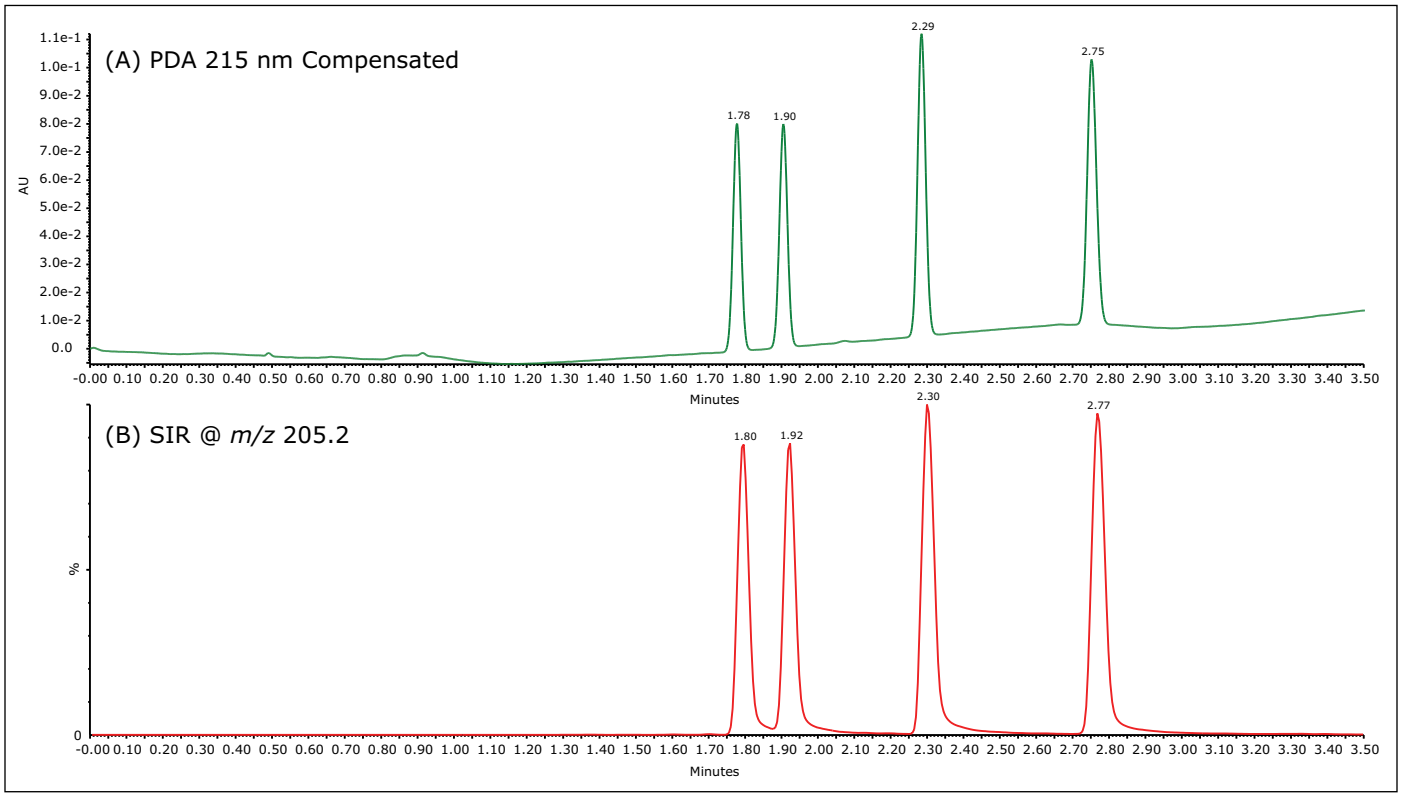

Figure 5. UPC^2 chromatograms of a nerolidol standard separated on an ACQUITY UPC^2 Trefoil AMY1 Column: (A) UV at 215 nm with a compensation wavelength of 260-310 nm; and (B) SIR at m/z 205.2. The flow rate was 1.5 mL/min. A gradient of 2-7% isopropanol in 3.5 min was used.

CONCLUSIONS

In this application note, we have demonstrated the successful chiral separations of fragrance compounds on ACQUITY UPC^2 Trefoil AMY1 and CEL1 Columns using an ACQUITY UPC^2 System. The low system volume and extra-column volume of the UPC^2, combined with the reduced particle size of the ACQUITY UPC^2 Trefoil AMY1 and CEL1 Columns, enable superior, faster, and more efficient separations compared with traditional SFC and GC. The demonstrated analysis times range from 2 to 3 minutes, significantly shorter than the 15-50 minute analysis time typical for chiral GC,[3] allows for a fast authentication of the natural sources of essential oils. In all cases, the closely eluting isomers were baseline resolved. For the essential oil analysis, the oil samples were diluted and directly injected onto an ACQUITY UPC^2 System without tedious sample preparation. Furthermore, the inherent compatibility between UPC^2 and MS offered an unambiguous identification of the target analytes in a complex sample matrix. The high efficiency, short analysis time, and simple sample workup demonstrated in this study should be welcomed by industries where chiral analyses of fragrance compounds are routinely performed.

References

1. Leffingwell J, Leffingwell D. Chiral chemistry in flavours and fragrances. *Specialty Chemicals Magazine* 2010 March; 30-33.
2. Ravid U, Putievsky E, Katzir I, Weinstein V, Ikan R. Chiral GC analysis of (S)(+)- and (R)-carvone with high enantiomeric purity in caraway, dill and spearmint oils. *Flavour and Fragrance Journal* 1992; 7, 5, 289-292.
3. Konig W, Hochmuth D. Enantioselective Gas Chromatography in Flavor and Fragrance Analysis: Strategies for the Identification of Plant Volatiles. *Journal of Chromatographic Science* 2004; 44, 423-429.
4. Uzi R, Putievsky E, Katzir I, Raphael I. Determination of the enantiomeric composition of terpinen-4-ol in essential oils using a permethylated β-cyclodextrin coated chiral capillary column. *Flavour and Fragance Journal* 1992; 7, 1, 49-52.
5. Yaguchi, Y. Enantiomer separation of fragrance compounds by supercritical fluid chromatography. Seibutsu Kogaku Kaishi 2010; 88, 10 520-524.
6. Sugimoto D, Yaguchi Y, Kasuga H, Okajima S, Emura M. Preparation of chiral flavor chemicals using enatioselective supercritical fluid carbon dioxide chromatography. Recent Highlights in Flavor Chemistry and Biology, Proceedings of the 8th Wartburg Symposium on Flavor Chemistry and Biology. Eisenach, Germany, February 27-March 2, 2007, 340-344.
7. Martin D, Gershenzon J, Bohlmann J. Induction of volatile terpene biosynthesis and diurnal emission by methyl jasmonate. *Plant Physiology* 2003; 132, 3, 1586-1589.

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ACQUITY UPLC PDA Analysis of Biocides (Part 1)

Peter J. Lee and Alice J. Di Gioia
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Improves laboratory productivity by enabling the rapid and sensitive separation of six commonly used biocides.
- Library matching and quantification automated with Empower® 3 Software increases confidence in peak confirmation and helps ensure product quality.
- Suitable for cosmetic and personal care product development and quality control analytical testing.

WATERS SOLUTIONS

ACQUITY UPLC® System

ACQUITY UPLC PDA Detector

Empower 3 Chromatography Data Software

ACQUITY UPLC BEH C_{18} Column

KEY WORDS

Biocides, cosmetics, personal care products, consumer products, product development, quality control, regulatory compliance, library matching

INTRODUCTION

Unwanted micro-organisms such as bacteria, viruses, and molds can grow wherever there is a source of nutrition and moisture. This unwanted growth may negatively impact human health, interfere with manufacturing processes, damage building structures, and spoil consumer goods. The principal defense against deleterious micro-organisms is biocides, commonly classified as disinfectants, preservatives, antifouling products, and pest controls.

Biocides are used as additives in cosmetics and personal care, household, and industrial products. To protect the environment and human health, many countries regulate biocide use.[1] In the European Union, this is done through the Directive 98/8/EC (The Biocidal Products Directive) and Regulation (EU) No 528/2012 (The Biocidal Products Regulation). In the United States, regulatory control of biocides falls under the EPA and the biocides applications in cosmetics, food, and personal health care are regulated by the U.S. FDA. Regulations impact the registration, labeling, and composition of biocides.[2] Reliable and rapid methods are therefore essential to ensure effective product quality control. This application note describes a three-minute separation of six biocides using the Waters® ACQUITY UPLC PDA System with Empower 3 Software. With PDA library matching and the built-in advanced mathematical algorithms, each biocide in the mixture can be identified and quantified; the analysis is fast and reproducible. The ability to quickly and unambiguously analyze biocide content can facilitate workflow related to the quality control and regulatory compliance of biocide containing products. This methodology benefits new product development, product troubleshooting and biocide production.

EXPERIMENTAL

Sample preparation

Analytes are:

Kathon CG/ICP
[containing 0.4% of **2-methyl-4-isothiazolin-3-one** (1a),
1.2% of **5-chloro-2-methyl-4-isothiazolin-3-one** (1b)];

Carbendazim (2);

Benzisothiazol-3(2H)-one (3);

2-phenoxyethanol (4);

Benzoic Acid (5);

Methyl paraben (6).

LC conditions

LC system:	ACQUITY UPLC PDA
Software:	Empower 3
Weak wash:	95:5 Water: CH_3CN (600 µL)
Strong wash:	50:50 Water: CH_3CN (200 µL)
Seal wash:	90:10 Water: CH_3CN (5 min)
Column temp.:	30 °C
Flow rate:	1 mL/min
Injection:	5 µL
Detection:	PDA 210 to 500 nm
Sampling rate:	20 pts/s
Filter response:	0.1 s
Column:	ACQUITY UPLC BEH C_{18} 2.1x 50 mm
Mobile phase A:	0.05 v% Trifluoroacetic acid (TFA) in water
Mobile phase B:	0.05 v% TFA in CH_3CN
Linear gradient:	5% to 15% B in 2.9 min

Note: The column was equilibrated with 5% B for 2 minutes before injection, and was washed with 100% B for 2 minutes at the end of each run.

RESULTS AND DISCUSSION

Figure 1 shows the structures of the biocides (1a, 1b, 2-6); a 5 ppm mixture of 1–6 was separated using the Waters ACQUITY UPLC System with a three-minute linear gradient method. These compounds are frequently used in adhesives, paint and coatings, latex and sealants, inks, wood and paper products, textile and leather products, metalworking fluids, personal care products, cosmetics, laundry detergents, dishwashing liquids, and household and industrial cleaners. The acetonitrile/ water mobile phase with TFA modifier is compatible with mass spectrometry detectors, if needed.

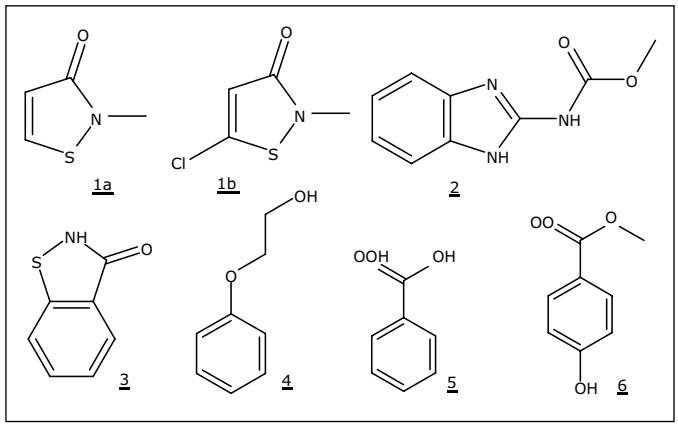

Figure 1. Chemical structures of biocides.

UV photodiode array (PDA) detection combined with Empower 3 Software enables a powerful range of detection and identity confirmation possibilities for chromatographic separations. PDA timed wavelength chromatograms can be plotted using the λmax of each analyte. This increases the detection limit when the analytes have very different λmax and aids quantification. Figure 2 is an overlay of nine replicate injections of PDA timed wavelength chromatograms, demonstrating that the overall reproducibilty is excellent. The three-minute linear gradient easily resolves the two active components of Kathon CG/ICP (1a and 1b) and the other five biocides. The retention time and peak area of each component observed in the above nine replicate injections are listed in Tables 1 and 2, with statistical analysis results generated using Empower 3 Software. The excellent % RSD results indicate the suitability of UPLC with BEH column chemistry for biocides.

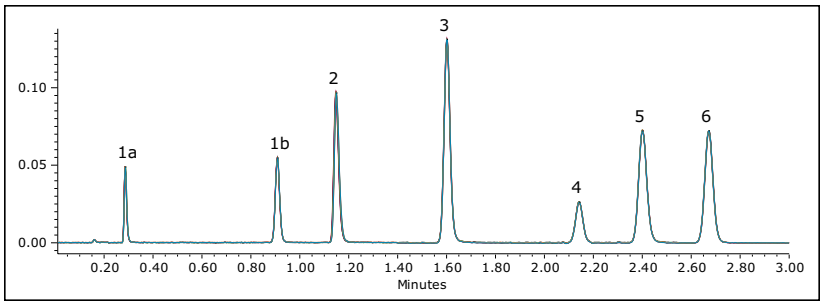

Figure 2. Overlay PDA timed wavelength chromatograms, retention time and peak area tables of 9 replicate injections of sample containing 1.25 ppm of 1a, 3.75 ppm of 1b and 5 ppm of 2–6: (0.00 min, 275 nm; 1.40 min, 225 nm; 2.55 min, 255 nm).

	1a (min)	1b (min)	2 (min)	3 (min)	4 (min)	5 (min)	6 (min)
1	0.286	0.908	1.147	1.600	2.141	2.400	2.671
2	0.287	0.908	1.147	1.601	2.141	2.400	2.671
3	0.286	0.908	1.147	1.601	2.141	2.401	2.672
4	0.286	0.908	1.148	1.602	2.142	2.401	2.672
5	0.286	0.908	1.148	1.601	2.141	2.400	2.671
6	0.286	0.908	1.149	1.602	2.142	2.401	2.672
7	0.287	0.908	1.148	1.601	2.141	2.400	2.671
8	0.286	0.908	1.150	1.602	2.142	2.401	2.672
9	0.287	0.909	1.150	1.602	2.142	2.401	2.672
Mean	0.286	0.908	1.148	1.601	2.142	2.401	2.672
Std. Dev.	0.001	0.000	0.001	0.001	0.001	0.001	0.001
% RSD	0.19	0.04	0.10	0.03	0.03	0.02	0.02

Table 1. Component summary for retention time for 9 replicate injections of sample containing 1.25 ppm of 1a, 3.75 ppm of 1b and 5 ppm of 2–6: (0.00 min, 275 nm; 1.40 min, 225 nm; 2.55 min, 255 nm).

	1a	1b	2	3	4	5	6
1	34745	68748	134846	227719	57857	172510	173458
2	34684	69423	134511	227840	57682	170783	172192
3	34730	68894	134698	228692	57882	173440	172053
4	34741	69168	135187	228238	57388	173125	172113
5	34761	68952	134533	228331	58008	170433	172156
6	34673	69132	134817	228461	57802	170579	171725
7	34753	68903	135014	228616	57863	172557	171723
8	34781	68736	135018	227710	57845	170954	171833
9	34782	69050	134694	228489	57809	172072	172143
Mean	34739	69001	134813	228233	57793	171828	172155
Std. Dev.	38	219	229	383	174	1157	523
% RSD	0.1	0.3	0.2	0.2	0.3	0.7	0.3

Table 2. Component summary for area for 9 replicate injections of sample containing 1.25 ppm of 1a, 3.75 ppm of 1b and 5 ppm of 2–6: (0.00 min, 275 nm; 1.40 min, 225 nm; 2.55 min, 255 nm).

Six levels of calibration standards (containing Kathon and 2–6 from 2.5 to 20 ppm) were analyzed. Empower 3 Software has built-in mathematical features and functions. Calibration curves were created from the standards and the quantity of analyte in each sample was calculated automatically. Figure 3 shows the calibration plots generated by Empower 3, using the peak areas vs the concentration. The linearity of the calibration curves is excellent with the R^2 values (residual sum of squares) above 0.9999, except one with 0.9998. Table 3 shows a typical results analysis table for peak identification and quantification using a biocides standard mixture mix as a sample. The last column shows that amounts match well with actual values (1.25 ppm for 1a, 3.75 ppm for 1b, and 5 ppm for 2–6). The data suggest that UPLC/PDA is well suited to meet the regulatory demands for quantitative analysis of biocides.

Empower 3 Software provides the capability of creating a PDA library from pure component peaks in user chromatograms. Afterwards, the library matching and peak purity features can be used with samples to confirm peak identities and to give added confidence that spectrally distinct peaks are not-coeluting. Empower 3 uses Spectral Contrast™ theory to quantitatively compare the shapes of UV spectra during library matching and Peak Purity analysis.[3-6] Figure 4 shows UV spectra, extracted from PDA chromatograms of standards (1a, 1b, 2-6); these spectra were used to create a library with names and retention times. Table 3 shows an example of a default Empower Report table with PDA library matching and Peak Purity results. The values of Match Angle are smaller than Match Threshold and the values of Purity Angle are smaller than Purity Threshold, indicating that the analytes were well separated and matched with PDA library of biocides.

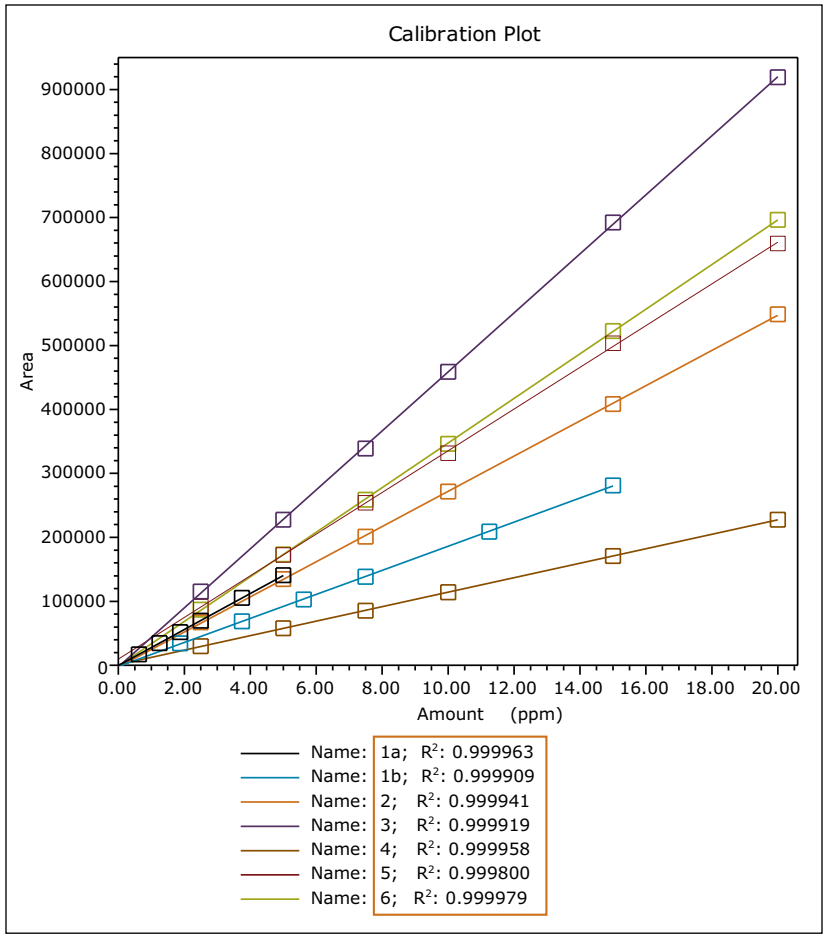

Figure 3. Calibration curves for (1a, 1b, 2-6).

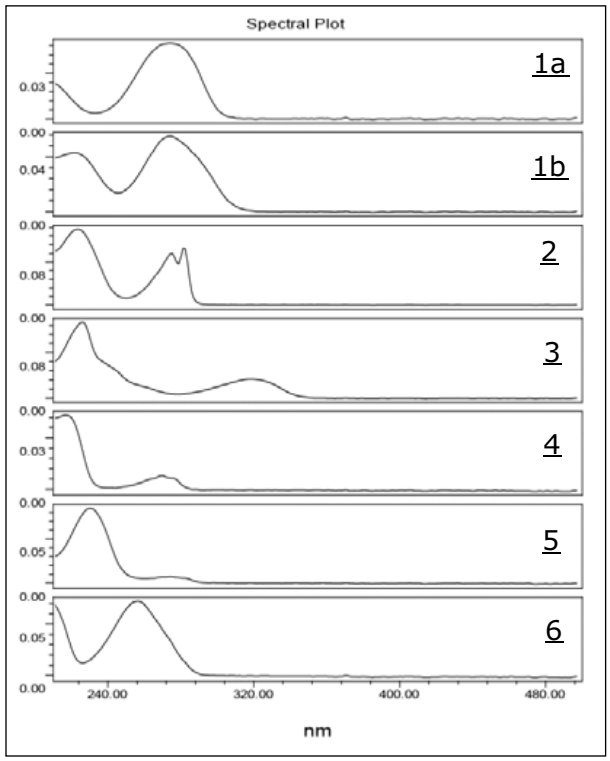

Figure 4. UV spectra of 1a, 1b, and 2-6 extracted from PDA data.

Component		Match1 Spect. Name	Match1 Angle	Match1 Threshold	Purity1 Angle	Purity1 Threshold	Amount (ppm)
1	1a	2-methy l-4-isothiazolin-3-one	0.312	1.598	0.792	1.086	1.25
2	1b	5-chloro-2-methy l-4-isothiazoline-3-one	0.401	1.474	0.676	0.863	3.78
3	2	carbendazim	0.184	1.244	0.337	0.554	5.01
4	3	1,2-benzisothiazol-3-one	0.174	1.282	0.368	0.628	5.01
5	4	2-phenoxyethanol	0.992	2.663	2.296	2.461	5.00
6	5	benzoic acid	0.322	1.399	0.560	0.760	4.99
7	6	methyl paraben	0.235	1.388	0.508	0.743	4.97

Table 3. Peak identification and quantification results shown on an Empower Report table, with additional PDA library matching and Peak Purity results.

CONCLUSIONS

Compliance with regulations that limit the type and concentration of biocides in a variety of applications necessitates analytical testing. This note illustrates that the Waters ACQUITY UPLC System with PDA detection enables rapid and sensitive separations of six commonly used biocides. With Empower 3 Software, library matching and quantification can be automated to add confidence in peak confirmation that is unavailabe with a single wavelength UV detector. This method is simple to use and suitable for quality control, new product development, and troubleshooting for both cosmetic and personal care manufacturers.

References

1. D J Knight, M Cooke. *The Biocides Business Regulation, Safety and Applications*, WILEY-VCH 2002,ISBN 3-527-30366-9.
2. United States Environmental Protection Agency. Antimicrobial Pesticide Registration. [cited 2015 November 9]. Available from: http://www2.epa.gov/pesticide-registration/antimicrobial-pesticide-registration.
3. M V Gorenstein, et al. Detecting Co-eluted Impurities by Spectral Comparison. LCGC. 12 (10): 768–772, 1994.
4. Peak Identification with Waters 996 Photodiode Detector: Library Matching, Waters Corporation, WPP12, Library No 960405.
5. Empower\Help\Interpreting PDA Peak Purity Results.
6. P J Lee, A J Di Gioia, ACQUITY UPLC/PDA: UV Filter Agents and Preservatives, Waters application note no. 720001770, 2006.

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ACQUITY UPLC PDA Analysis of Biocides (Part 2) Pass or Fail Custom Calculation

Peter J. Lee and Alice J. Di Gioia
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Increases laboratory productivity by enabling the high resolution, high sensitivity separations of biocides in three minutes.
- Helps improve decision-making, and ensure product quality by rapidly extracting and delivering critical QC data based on user criteria.

WATERS SOLUTIONS

ACQUITY UPLC® System

ACQUITY UPLC PDA Detector

Empower® 3 Chromatography Software

KEY WORDS

Biocides, cosmetics, personal care, user set criteria, custom calculations, custom field, Quality Control, pass or fail

INTRODUCTION

Making more informed decisions in less time is essential in today's Cosmetics, Personal Care, and Food analytical laboratories. Whether you work in method development, quality assurance, new product development, or testing for biocides, you seek a productivity edge. Waters® ACQUITY UPLC System provides that edge, enabling high resolution, high sensitivity biocide separations in three minutes.[1] Better quality information on product biocide levels can now be generated far more rapidly than with traditional HPLC methods. UPLC® combined with Empower 3 Software can effectively run separations, analyze data, and report results automatically. With the custom calculation function of Empower 3, raw data can be converted into the required format and the critical results can be used quickly by decision-makers to further enhance productivity. This application note describes the benefits of using a simple custom calculation created to determine if the biocide concentration in a sample passes or fails user set criteria. This type of custom calculation eliminates the need for manual calculation and prevents potential human errors. The ability to deliver critical information with speed and accuracy can help manufacturers reduce failed products, avoid product recalls and liability litigations. An example detailing the creation of a custom calculation shown in the note is provided in the Experimental section.

EXPERIMENTAL

QC Criteria Example for Biocide

Kathon amount < 4.85 ppm: Fail

Kathon amount > 5.15 ppm: Fail

Kathon amount from 4.85 ppm to 5.15 ppm: Pass

Create a Custom Field Calculation Method with Empower 3

1. Click Configure System to open the Configuration Manager window, click Projects in the tree.
2. Select and highlight the working project, then right click the highlighted project.
3. Select Properties to open Project Properties window.
4. Click the Custom Fields tab, then click New to open New Custom Field Wizard: Data and Type Selection window.
5. Select the Field Type: Peak, and Data Type: Enum, then click Next to open Source Selection window.
6. Select Data Source: Calculated, Sample Type: Unknown, Peak Type: Group Only, then click Next to open Formula Entry window (Figure 1).
7. In the Operations list, double-click **ENUM(** and **LT(.**
8. In the Fields list, double-click Amount, in the formula workspace, enter **,4.85),**
9. In the Operations list, double-click **LTE(.**
10. In the Fields list, double-click Amount, in the formula workspace, enter **,5.15).**
11. In the Operations list, double-click **GT(.**
12. In the Fields list, double-click Amount, in the formula workspace, enter **,5.15)).**
13. Click Next to open Translation Definition Table.
14. Enter **Fail** next to 0, **Pass** next to 1, **Fail** next to 2, click Next to open Name Entry window. (Note: you can enter Pass/Fail in another language).
15. Enter a name for the custom field in the Field Name text box: Pass or Fail, click Finish.

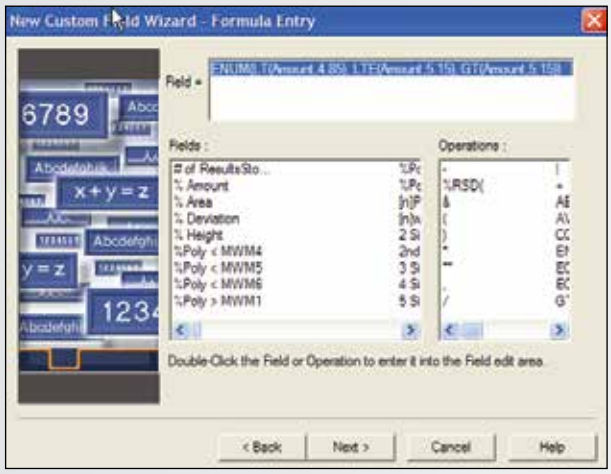

Figure 1. Formula Entry window.

Create Named Groups in the Processing Method

1. From Processing Method window, select the Named Groups tab.
2. Enter Kathon in the Name text box.
3. Select the option of Sum Peaks, Curve or Sum Peaks for Quantitation.
4. Drag 1a and 1b from Single Peak Components into the tree of Kathon as shown in Figure 2.

Sample preparation and UPLC Methods

The methods used are the same as described previously.[1] Analytes are Kathon CG/ICP [containing 0.4% 2-methyl-4-isothiazolin-3-one (1a), 1.2% 5-chloro-2-methyl-4-isothiazolin-3-one (1b)], Carbendazim (2), Benzisothiazol-3(2H)-one (3), 2-phenoxyethanol (4), Benzoic Acid (5), and Methylparaben (6).

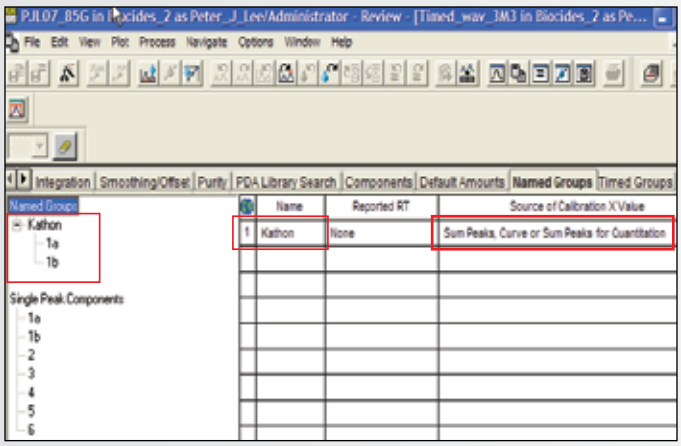

Figure 2. Processing method window.

RESULTS AND DISCUSSION

Figure 3 shows chromatograms of three biocide samples. There are seven well-resolved peaks in each sample. 1a and 1b are the two active ingredients of Kathon. The amount of 1a and 1b in each sample can be calculated automatically in Empower 3 Software from calibration curves as described previously.[1]

For this discussion, a passable QC level for the Kathon biocide is the range 4.85 ppm to 5.15 ppm. To determine Pass or Fail biocide status of each sample, the amount of total Kathon must be calculated, that is the sum of 1a and 1b, and the result must be compared with the QC criteria.

Figure 4 shows the Custom Field Formula described in the Experimental section. The QC Pass or Fail criteria is based on the Kathon biocide content (5 ppm ± 3%).

Using both the Named Groups and Custom Field calculation functions, Empower can be set up to automatically calculate and report the quantity of Kathon in each sample and determine if the sample met the QC criteria (Table 1). In an enterprise environment, the critical Pass or Fail results can be e-mailed to product and plant management. These advanced functions of Empower eliminate the need for manual calculations, which saves time and reduces errors.

	SampleName	Component	Amount (ppm)	Pass/Fail
1	PJL07_85G	Kathon	5.03	Pass
2	PJL07_85H_Low	Kathon	2.57	Fail
3	PJL07_85F_High	Kathon	7.43	Fail

Table 1. Biocides Test: Pass or Fail.

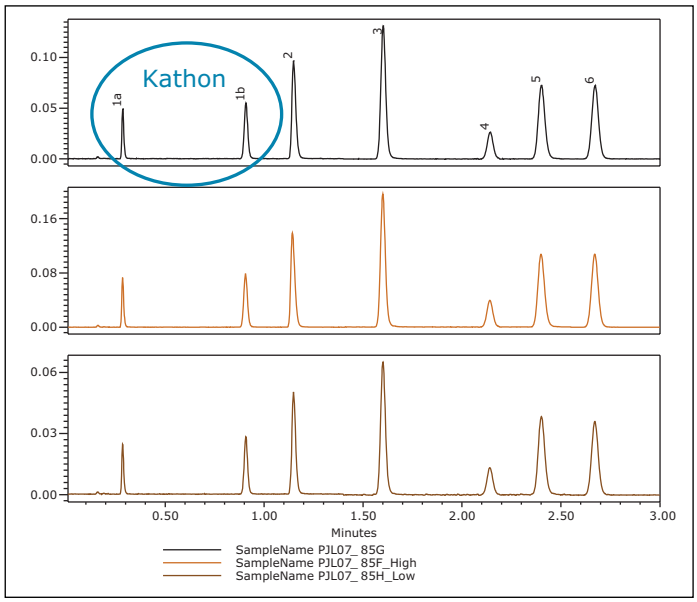

Figure 3. UPLC chromatograms of three biocide samples.

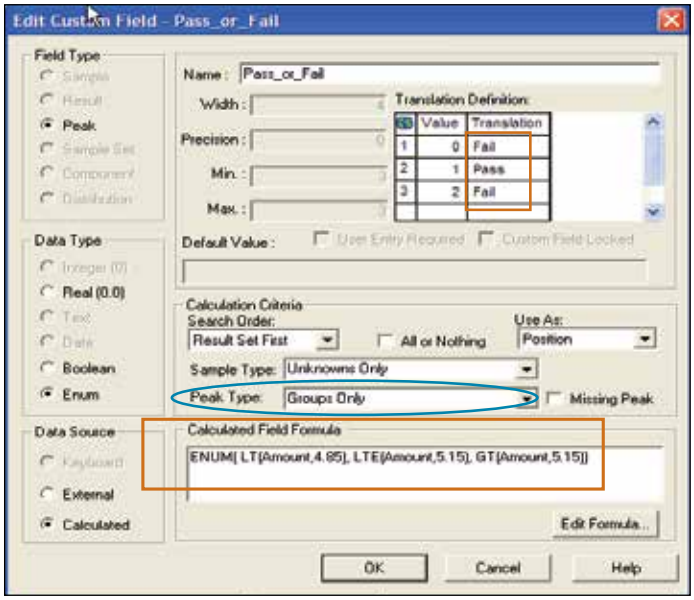

Figure 4. Custom Field formula.

CONCLUSIONS

Hundreds of biocide containing product samples can be automatically analyzed on a daily basis using the Waters ACQUITY UPLC PDA System with Empower 3 Software. Critical product QC information can be accurately extracted and rapidly delivered based on user set criteria. The method represents a significant productivity enhancement relative to the manual verification of QC biocide data. It can be very effective for food, cosmetics and personal care manufacturers, and formulators to have a report with a simple Pass or Fail for the sample displayed.

Reference

1. PJ Lee, AJ Di Gioia, *ACQUITY UPLC/PDA Analysis of Biocides (Part 1)*, Waters application note no. 720001832 2006.

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ACQUITY UPLC/PDA: UV Filter Agents and Preservatives

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APPLICATION BENEFITS

- Chromatographic methodology for rapid, reliable separation and confirmation of UV filter agents and preservatives.
- UV-based library matching of nine structurally similar sunscreens and preservatives.
- Easy-to-use experimental conditions suitable for raw material suppliers, cosmetics, and personal care product formulators.

WATERS SOLUTIONS

ACQUITY UPLC® System

Empower® 3 Chromatography Data Software

ACQUITY UPLC PDA Detector

ACQUITY UPLC BEH C_{18} Column

KEY WORDS

Library matching, sunscreen, cosmetics, personal care products, UV filter agents, raw materials, preservatives, consumer products, biocides

INTRODUCTION

UV filter agents and preservatives are widely used in a broad range of applications including cosmetics and personal care products, household products, plastics, paints, inks, and adhesives.[1-5] Worldwide government regulations and guidelines impact labeling, composition and registration of personal care, cosmetics and packaging products. It is important to use the best science and instrumentation to evaluate not only the final products but the ingredients as well. In the United States, 9 UV-B filter agents and 7 UV-A filter agents have FDA approval for use in sunscreen formulations.[6] Whereas, 28 UV filter agents are permitted for sunscreens in Europe.[6]

Formulators of sunscreen products in the U.S. must be in compliance with FDA regulations. It benefits both the chemical manufacturers of UV filter agents and the formulators to verify the identity and purity of these organic UV filters.

Preservatives, the biocides used in cosmetics and personal care products to prevent bacteria, mold, and other contaminants. To protect the environment and human health, many countries regulate biocide use. In the European Union, this is done through the Directive 98/8/EC (The Biocidal Products Directive) and Regulation (EU) No 528/2012 (The Biocidal Products Regulation). In the United States, regulatory control of biocides falls under the EPA and the biocides applications in cosmetics, food, and personal health care are regulated by the U.S. FDA.

In the United States, more than 50% of preservatives used in personal care products are parabens, iosthiazolinones, and formaldehyde donors such as imidazolidinyl urea. With common preservatives such as parabens coming under greater scrutiny due to regulatory and consumer perception issues,[7,8] manufacturers find themselves defending the use of these additives or searching for substitutes.

This application note describes a seven minute separation and identification of nine structurally similar sunscreens and preservatives using the Waters® ACQUITY UPLC/PDA System with Empower 3 Software and library matching.

EXPERIMENTAL

Sample preparation

Analytes **1–9** (Figure 1) were dissolved in CH_3CN to make 100 µg/mL stock solutions:
2-Phenoxyethanol [122-99-6], **1**;

Benzoic acid [65-85-0], **2**;

Methylparaben [99-76-3], **3**;

Propylparaben [94-13-3], **4**;

Oxybenzone [131-57-7], **5**;

Avobenzone [70356-09-1], **6**;

Octinoxate [5466-77-3], **7**;

Octisalate [118-60-5], **8**;

Homosalate [118-56-9], **9**.

The working solution (50 µg/mL) was prepared by mixing 500 µL of the stock solution with 500 µL D.I. H_2O.

UPLC conditions

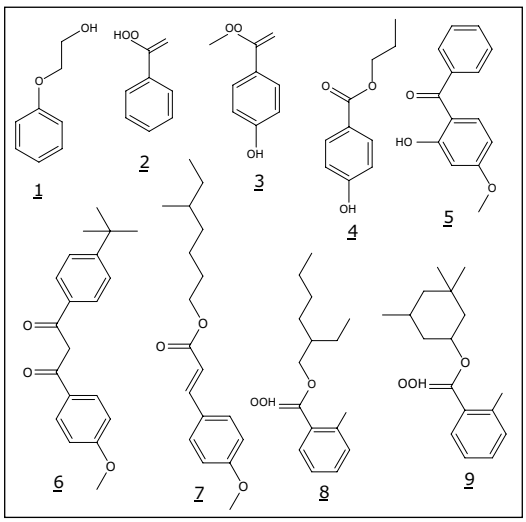
UPLC system:	ACQUITY UPLC
Software:	Empower 3
Weak wash:	95:5 Water: CH_3CN (1 mL)
Strong wash:	CH_3CN (1 mL)
Seal wash:	90:10 Water: CH_3CN (5 min)
Column temp.:	50 °C
Flow rate:	0.8 mL/min
Injection:	3 µL
Detection:	PDA 215 to 500 nm
Sampling rate:	20 pts/s
Filter response:	0.1 s
Linear gradient:	5% B to 100% B in 7 min
C_{18} column:	ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm
Mobile phase A:	0.05 v% of TFA in H_2O
Mobile phase B:	0.05 v% of TFA in CH_3CN

Traditionally, HPLC is used to analyze biocides and UV filter agents with a typical run time of 20 to 50 minutes.[1-5] In contrast, the ACQUITY UPLC System can provide a rapid, reliable separation and confirmation of the target organic compounds in less than 10 minutes. This can facilitate workflow for both raw material suppliers and personal care product formulators in quality control, regulatory compliance, new product development, and product troubleshooting.

RESULTS AND DISCUSSION

Figure 1 shows the chemical structures of four preservatives (**1–4**) and five UV filter agents (**5–9**) discussed in this note. These compounds are among the most commonly used biocides and organic sunscreens in personal care and cosmetic products.[6-8] A mixture of **1–9** was separated using a ACQUITY UPLC System with a 2.1 x 100 mm BEH C_{18} Column using a seven minute linear gradient method (5% B to 100% B). The solvents employed for the separation are common, easy to prepare and suitable for use with mass spectrometry detectors, if needed: 0.05 v% TFA in H_2O (mobile phase A) and 0.05 v% TFA in CH3CN (mobile phase B).

UV photodiode array (PDA) detection combined with Empower 3 Software enables a powerful range of detection and identity confirmation possibilities for chromatographic separations. PDA timed wavelength chromatograms were plotted using the λmax of each analyte. This can increase the detection limit when the analytes have very different λmax and aid quantification. Figure 2 shows an overlay of 12 replicate injections of PDA timed wavelength chromatograms.

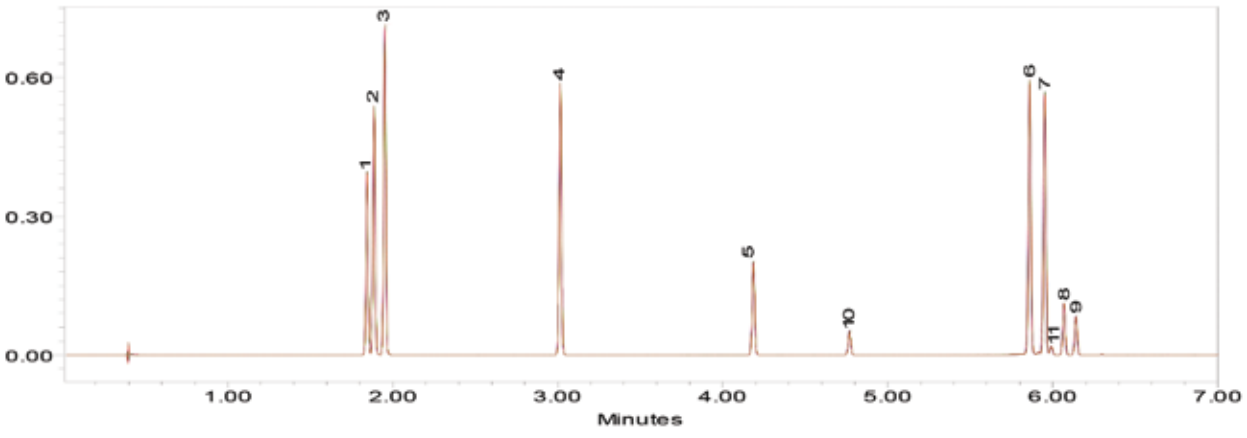


Chemical structures of four preservatives (1–4) and five UV filter agents (5–9).

Visual examination shows the overall reproducibilty is excellent. Despite the similar groups of chemical structures, the components are well-resolved by the 7-minute linear gradient method. Two impurities in the mixture that previously co-eluted are now separated, as shown in Figure 2. Peak **10** is an unknown impurity in the avobenzone (6) standard whereas peak 11 is an isomer of homosalate (**9**).

The Empower 3 report table in Figure 2 shows that the %RSD ranges from 0.02 % to 0.04%. Retention time reproducibility is a good indicator of the robustness and suitability of UPLC with BEH Column chemistry for preservatives and sunscreens.

To confirm peak identities and provide assurance regarding spectral peak purity or "non-coelution" a user can build a PDA library and perform library matching and peak purity analysis through Empower 3 Software.[9,10]



Component Summary For Retention Time

	SampleName	1 (min)	2 (min)	3 (min)	4 (min)	5 (min)	10 (min)	6 (min)	7 (min)	11 (min)	8 (min)	9 (min)
1	9_analytes_50ug	1.844	1.888	1.953	3.017	4.187	4.770	5.862	5.953	5.993	6.070	6.142
2	9_analytes_50ug	1.845	1.888	1.953	3.017	4.187	4.770	5.861	5.953	5.993	6.070	6.142
3	9_analytes_50ug	1.845	1.888	1.953	3.017	4.187	4.770	5.862	5.953	5.993	6.070	6.143
4	9_analytes_50ug	1.844	1.887	1.952	3.016	4.186	4.769	5.860	5.951	5.991	6.068	6.141
5	9_analytes_50ug	1.844	1.886	1.951	3.016	4.186	4.768	5.860	5.951	5.991	6.068	6.141
6	9_analytes_50ug	1.842	1.885	1.950	3.014	4.184	4.767	5.858	5.949	5.989	6.066	6.138
7	9_analytes_50ug	1.843	1.886	1.951	3.015	4.185	4.768	5.859	5.950	5.990	6.067	6.139
8	9_analytes_50ug	1.844	1.887	1.952	3.016	4.186	4.769	5.860	5.951	5.991	6.068	6.141
9	9_analytes_50ug	1.845	1.887	1.952	3.017	4.187	4.770	5.860	5.952	5.992	6.069	6.141
10	9_analytes_50ug	1.845	1.887	1.953	3.017	4.187	4.770	5.861	5.952	5.992	6.069	6.142
11	9_analytes_50ug	1.845	1.888	1.953	3.017	4.188	4.769	5.860	5.951	5.991	6.068	6.141
12	9_analytes_50ug	1.844	1.886	1.952	3.016	4.185	4.768	5.859	5.950	5.990	6.067	6.139
Mean		1.844	1.887	1.952	3.016	4.186	4.769	5.860	5.951	5.992	6.068	6.141
Std. Dev.		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
% RSD		0.043	0.044	0.043	0.026	0.023	0.020	0.021	0.021	0.021	0.021	0.021

*Figure 2. Overlay PDA timed wavelength chromatograms and retention time table of 12 replicate injections of **1–9** : (0.00 min, 220 nm), (1.92 min, 250 nm), (5.0 min, 360nm), (5.9 min, 300 nm).*

Figure 3 shows UV spectra extracted from PDA chromatograms of standards (**1–9**) that were used to create a library with names, concentrations, and retention times. Empower 3 uses Spectral Contrast theory to quantitatively compare the shapes of UV spectra during library matching and peak purity analysis. The match angle or purity angle indicates how closely the spectra overlap. A spectral contrast angle of 0° means that the spectra overlay perfectly and the compounds these spectra represent are identical; a 90° angle means that the two spectra do not overlap and that the compounds are different.

The Threshold Angles are an indication of "uncertainty" or non-idealities. If the Match or Purity (Spectral Contrast) Angle is less than the Match or Purity Threshold Angle, this indicates that the differences between the spectra are from non-idealities and the match is "good" or the peak is spectrally pure. If the Spectral Contrast Angle is greater than the Threshold Angle, then the differences are due to true differences between the spectra. After a library is available, the library matching and peak purity process can be automated in Empower for identification and peak purity confirmation.

Table 1 provides an example of a default Empower table with PDA library matching and peak purity results. The values Match Angle and Purity Angle indicate that the UV-filter agents and biocides were well matched with PDA library of sunscreen agents and preservatives.

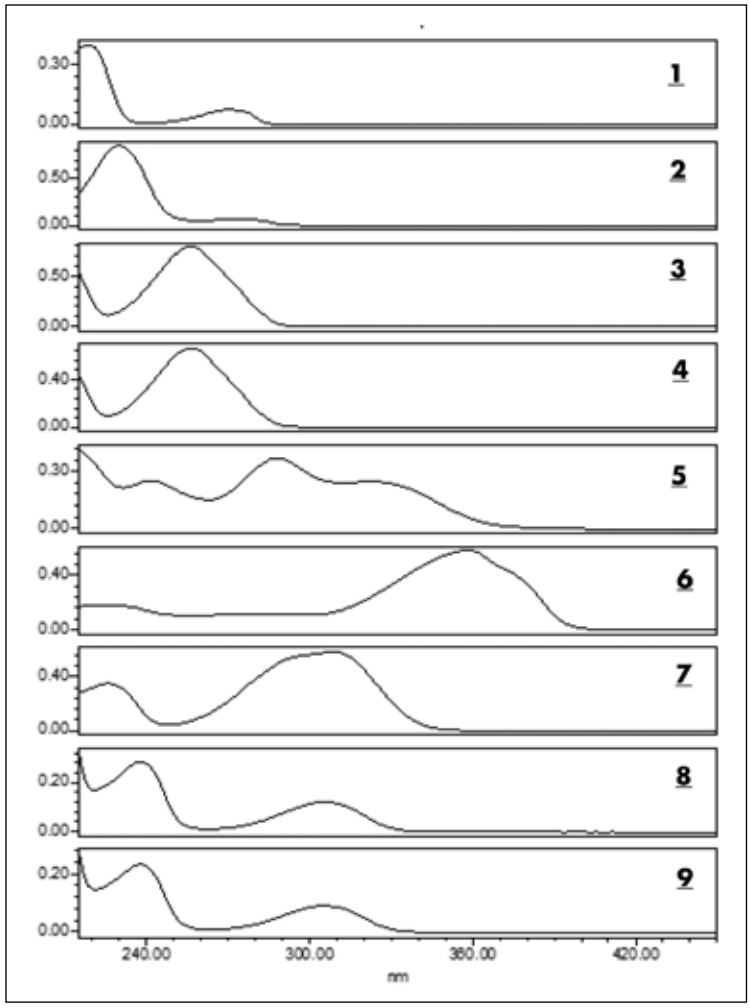


*Figure 3. UV spectra extracted from PDA chromatograms of standards (**1–9**).*

	Name	RT	Match1 Spect. Name	Match1 Angle	Match1 Threshold	Purity1 Angle	Purity1 Threshold
1	1	1.845	Phenoxyethanol	0.290	1.497	0.359	0.407
2	2	1.888	Benzoic acid	0.103	1.112	0.069	0.302
3	3	1.953	Methyl paraben	0.060	1.036	0.046	0.289
4	4	3.017	Propyl paraben	0.058	1.043	0.056	0.290
5	5	4.187	Oxybenzone	0.056	1.064	0.067	0.291
6	6	5.862	Avobenzone	0.095	1.061	0.132	0.315
7	7	5.953	Octinoxate	0.082	1.045	0.088	0.287
8	8	6.070	Octisalate	0.167	1.171	0.142	0.336
9	9	6.142	Homosalate	0.156	1.218	0.156	0.366

Table 1. PDA library matching results for peak identification.

CONCLUSIONS

The Waters ACQUITY UPLC System with PDA Detection and Empower 3 Software provide sensitive, baseline resolved, rapid separations with automated library matching. This has been demonstrated with a rapid, reproducible separation of a mixture of nine of the most commonly used organic UV sunscreens and biocides in cosmetics and personal care products. The easy-to-use experimental conditions are suitable for raw material suppliers, cosmetics, and personal care product formulators. Applications include quality control, new product development, and troubleshooting.

References

1. L H Wang, *Chromatographia.* 50*:* 565–570, 1999.
2. M E Balmer et al. *Environ Sci Tech*. 39: 953–962, 2005.
3. V Vanquerp et al. *J Chromatogr A*. 832: 273–277, 1999.
4. M Frauen et al. *J Pharm Biomed Anal*. 25: 965–970, 2001.
5. E Marengo et al. *J Chromatogr A*. 1029: 57–65, 2004.
6. M S Reisch. New Wave Sunscreens. *C&EN*. 83(15): 18–22, 2005.
7. M S Reisch. New Wave Sunscreens. *C&EN*. 83(15): 1–22, 2005.
8. D deGuzman. *Chemi Mkt Reporter. 268 (19): 20–22, 2005.*
9. M V Gorenstein, et al. *LC-GC*. 12: 768–772, 1994.
10. Peak Identification with Waters 996 Photodiode Detector: Library Matching, Waters Corporation, WPP12, Library No. 960405, 1995.

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COUNTERFEIT DETECTION

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Application of Multivariate Analysis and LC-MS for the Detection of Counterfeit Cosmetics

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APPLICATION BENEFITS

- A rapid, simple method using high-resolution mass spectrometry and multivariate analysis to compare authentic cosmetics samples with suspected counterfeit samples.
- Can be adopted for comparative analysis of cosmetic products samples, as well as for other types of analysis where an evaluation of differences is needed (e.g. failed batch of raw materials or packaging).

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

CORTECS® UPLC C_{18} Column

UNIFI® Scientific Information System

Xevo® G2-XS QTof Mass Spectrometer

KEY WORDS

Counterfeits, cosmetics, multivariate analysis, accurate mass QTof, packaging analysis

INTRODUCTION

Every year the cosmetics industry suffers multi-billion dollar losses due to counterfeit cosmetic products.[1] Due to much stricter regulatory controls in Europe and North America, 66% of counterfeit goods come from Asia.[2] The risk to the consumer is high, not because they are paying for a counterfeit product, but because the ingredients used in the production of counterfeit cosmetics could be harmful to their health, or even banned for human use.

Testing for counterfeit products is occasionally done by the cosmetics companies – especially those companies whose high-end products are usually the target of counterfeiting, since only they know the correct formulation of their products. However, it would be beneficial and less time consuming if counterfeit testing could be done at the point of entry in the country, for instance, during customs inspection. Even if the correct formulation is not known, it is possible to compare suspected fake samples with authentic samples using multivariate statistical analysis and assess the differences if needed.

Multivariate analysis (MVA) is widely used in the areas where multiple samples or batches need to be compared. One of the most commonly used techniques is principal component analysis (PCA) which allows the reduction of a large set of multivariate data into uncorrelated variables called principal components.

EXPERIMENTAL

Sample preparation

High-end cosmetic samples were purchased from the U.S. manufacturer. A cream, a lotion, and a serum were chosen for this study. Identical looking items were resourced from an online retailer in Asia. All samples were prepared by dilution in tetrahydrofuran (THF) at a concentration of mg/mL.

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class
Separation mode:	Gradient
Column:	CORTECS UPLC C_{18}, 90Å, 1.6 µm, 2.1 mm x 100 mm
Column temp.:	40 °C
Injection volume:	5 µL
Flow rate:	0.4 mL/min
Mobile phase A:	0.1 % formic acid in water
Mobile phase B:	0.1 % formic acid in methanol
Gradient:	20% B held for 30 s, increased to 99% over 2.5 min, held at 99% for 6 min, then re-equilibrated back to 20%

MS conditions

MS system:	Xevo G2-XS QTof
Ionization mode:	ESI + and -
Capillary voltage:	3.0 kV for pos, 2.0 for neg
Desolvation temp.:	450 °C
Source temp.:	150 °C
Cone voltage:	30 V
Collision ramp:	10 to 40 eV
MS scan range:	50 to 1200 *m/z*

Data acquisition and processing

Waters® UNIFI Scientific Information System was used for data acquisition and processing.

RESULTS AND DISCUSSION

Samples were analyzed as five replicates in positive and negative ESI mode to obtain a representative data set in both ionization modes. Due to the lack of information about the counterfeit sample formulations, and for a more comprehensive analysis, data was acquired using different ionization modes as some compounds will ionize exclusively with positive ionization, and others only by negative ESI.

All sample data was processed using the multivariate analysis tools available in UNIFI Scientific Information System. UNIFI can generate marker matrices based upon user-defined criteria that can be automatically transferred to EZInfo software for MVA. The initial summary is presented as a PCA scores plot. In this initial plot no information about the individual sample groups is passed to the MVA software, and this model is said to be unsupervised.

If additional discrimination among the investigated sample groups is required, a supervised analysis model, such as the Projection to Latent Structures Discriminant Analysis (PLS-DA) model (Figure 1) can be employed. PLS-DA models the quantitative relationships between the variables X (predictors) and Y (responses) for all the sample groups and can be used to elucidate group differences. However, in these types of plots, each sample is presented by a single point, which does not allow individual markers contributing to the differences between the groups to be observed.

In Figure 1a, the data obtained by ESI- is presented. It can clearly be seen that there are differences between each of the samples. In this plot a general trend can be observed that the authentic product samples fall in the lower quadrant, biased toward the left side, while the counterfeit samples appear at the top and toward the right.

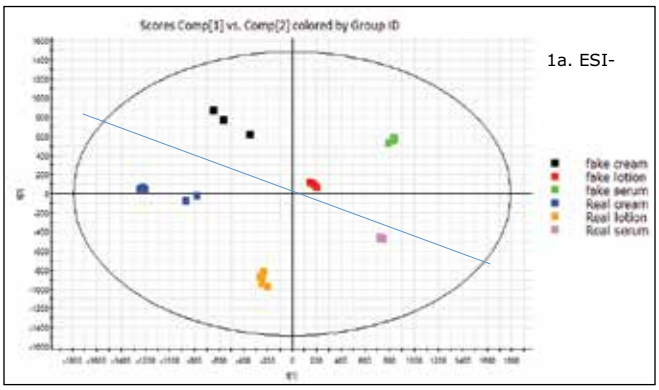

Figure 1a. PLS-DA plot for all the samples in ESI- mode.

For clarity, a blue line has been added to the plot showing the counterfeit data above the blue line, and all the authentic product samples below it. This plot indicates that there is some element of the data that is common in the ESI- and contributes to the grouping of the two sets of samples. Although significant differences are also seen in positive ion mode (Figure 1b), no general trend for counterfeit versus authentic samples was observed.

Each of the sample groups (creams, lotions and serums) were further investigating by using Orthogonal Projection to Latent Structures – Discriminant Analysis (OPLS-DA) scores plots, shown in Figure 2.

OPLS allows analysts to mine the data for additional information beyond that of simple differences between groups. This additional level of detail is needed to identify specific features of the data that contribute to what makes the samples different from one another, such as to discover whether the difference in the counterfeit samples is due to chemicals that are harmful to human health. The tool used to dig deeper into the data is called an S-plot. The S-plot shows the Accurate Mass/Retention Time (AMRT) dissimilarities between these two groups, shown in Figure 3.

The AMRT pairs are plotted by covariance – the magnitude of change (x-axis), and correlation – the consistency of the change (y-axis) values. The upper right quadrant of the S-plot shows AMRTs which are elevated in the authentic sample, while the lower left quadrant shows components elevated in the counterfeit sample. In this case, an AMRT may represent a component of the formulation that is different between the two samples. The farther along the x-axis the marker is located, the greater its contribution to the variance between the groups, while markers farther along the y axis represent a higher reliability of the analytical result. The differences between the groups can come from analytes which are not present in one of the groups, or from analytes with the greatest change in intensity (concentration) between the groups.

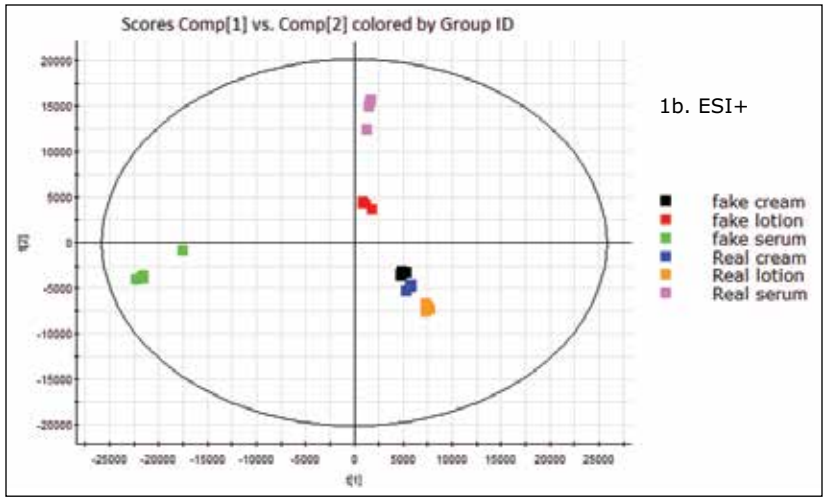

Figure 1b. PLS-DA plot for all the samples in ESI+ mode.

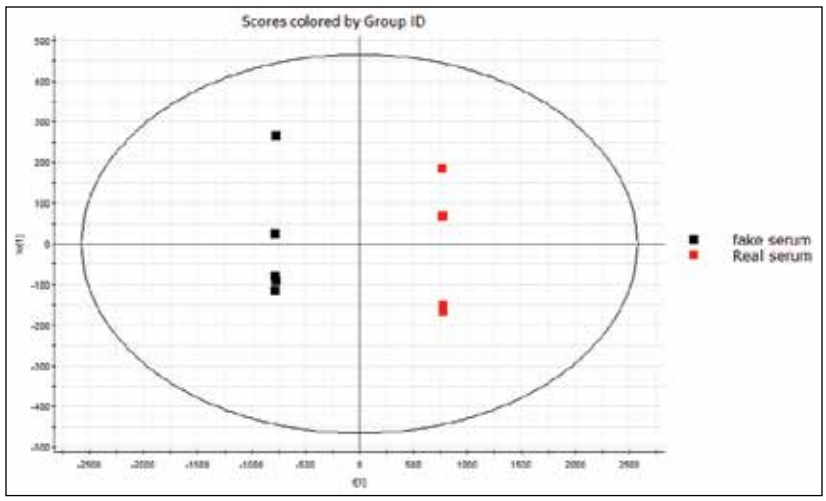

Figure 2. OPLS-DA plot for serum samples in ESI- mode.

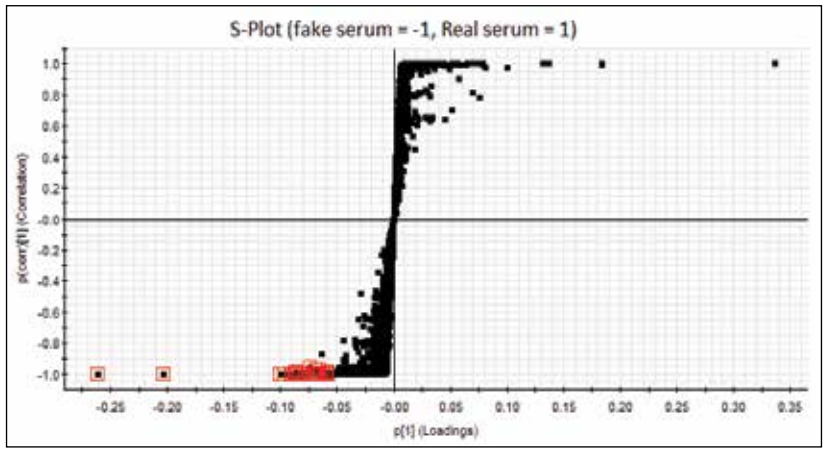

Figure 3. S-plot for counterfeit and authentic serum samples in ESI- mode. Markers selected in red have the greatest contribution to the variance between the fake serum and the authentic one.

After selecting the markers which contribute to the differences between the groups, each marker set can be labeled accordingly. For example, markers specific to the differences between fake cream and the authentic cream. The labels can be appended if the marker is found in more than one selection. Such group comparison was done for all three types of samples. After labeling each group, it was observed that two markers were present in all three types of counterfeit samples – *m/z* 151.0409 at 2.59 mins, and *m/z* 179.0725 at 3.64 mins (Figure 4). These two AMRTs very likely contributed towards the distinct separation observed in the PLS-DA (ESI-) plot between the authentic samples and the counterfeits. In the trend plot, it was also observed that these markers were not detectable in the authentic samples.

To investigate the markers further, the discovery tools in the UNIFI Scientific Information System were employed. Both markers were submitted for automated elemental composition calculation, structural database search, and fragment matching of the high collision energy data. The results are shown in Figure 5.

For the first marker a molecular formula of $C_8H_8O_3$ was proposed. From the corresponding structures in the ChemSpider database, methylparaben and methyl salicylate have most high collision energy fragments matched (2). The second marker has a molecular formula of $C_{10}H_{12}O_3$ and propylparaben and 4-propoxybenzoic acid have the most fragments matched (4). Methyl salicylate is used as a fragrance in foods and beverages. 4-propoxy benzoic acid can be used in chemical synthesis of liquid crystals. Instead, parabens are preservatives most commonly used in personal care products like body lotions and creams.[3] However, due to public awareness and concerns about parabens being endocrine disruptors, high-end cosmetics companies have stopped using them in their products.[4,5]

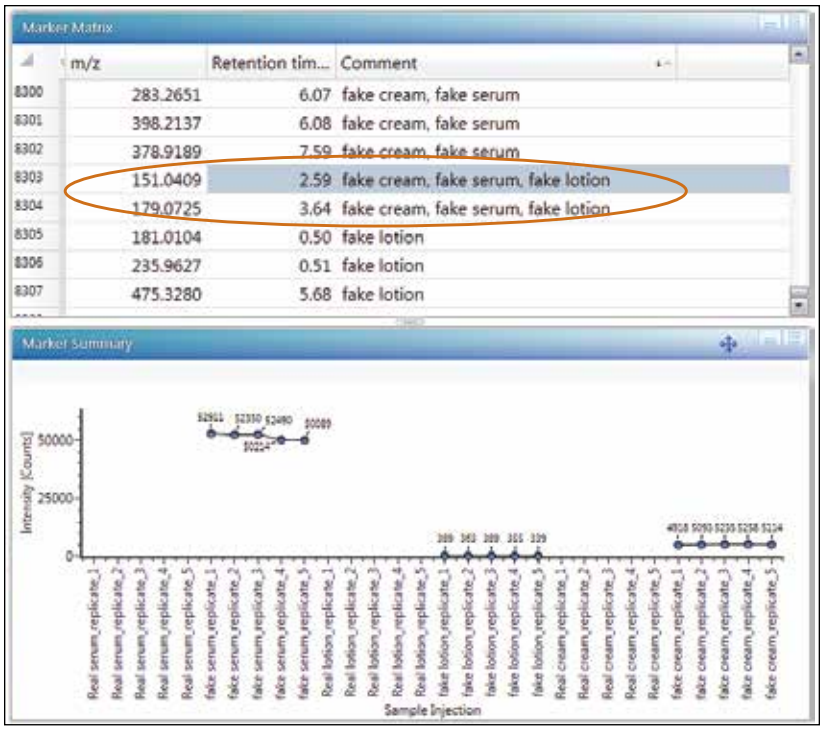

Figure 4. Marker table and the trend plot for ESI- data.

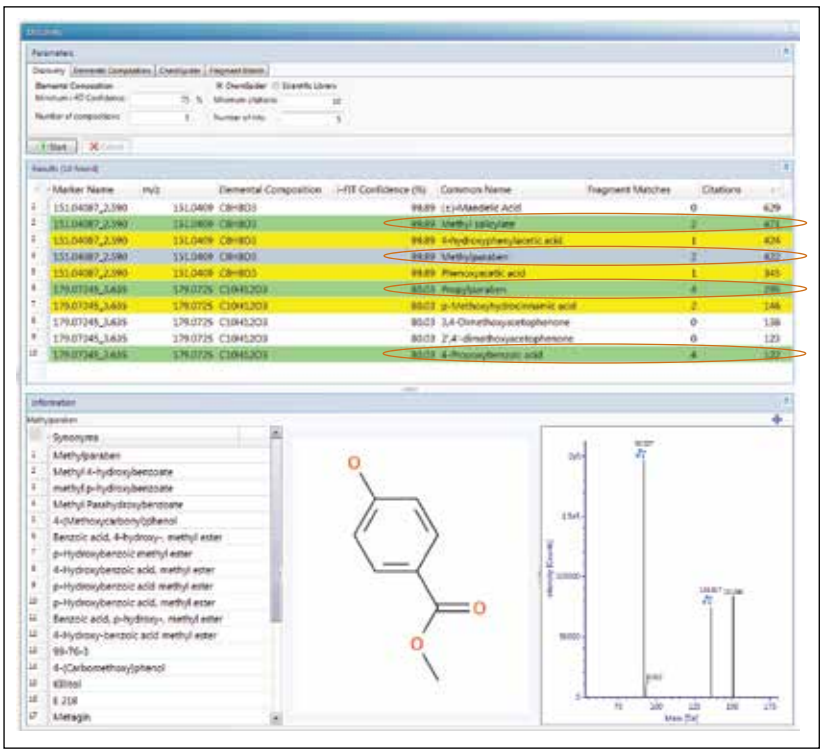

Figure 5. Discovery tool results for m/z 151.0409 and 179.0724. Summary of elemental compositions, citations for structures retrieved from ChemSpider database and a number of possible fragment matches in high collision energy data for each structure.

The producers of the counterfeit products have no such concern, and in this case, appear to have formulated the product using the lowest cost chemicals available to obtain a product which superficially appears the same as an authentic sample.

Based on information available from the data and discovery tools, together with the information available on the chemicals proposed, there is high confidence in the assignment of parabens to these two markers at *m/z* 151.0409 and 179.0724.

CONCLUSIONS

Every year the cosmetics industry suffers from multi-billion dollar losses due to counterfeit cosmetics products. This lost revenue may have a negative impact on market share, and can result in a further erosion of sales. If the counterfeit products cause health problems in consumers, this can damage the reputation and brand image for the manufacturers of the authentic cosmetics. Early and rapid detection of counterfeit products is one way to address counterfeiting in both domestic and export markets. Highlighted in this work is a multivariate analysis technique for sample comparison using statistical analysis tools for easy comparison between complex samples. The described LC-MS and informatics workflow as implemented with the UNIFI Scientific Information System using high-resolution mass spectrometry can be adopted for cosmetics, food and beverage, and pharmaceutical sample analysis.

References

1. https://oami.europa.eu/ohimportal/en/web/observatory/news/-/action/view/1934074
2. www.ccapcongress.net/archives/Brussels/Files/fsheet5.doc
3. http://www.fda.gov/Cosmetics/ProductsIngredients/Ingredients/ucm128042.htm
4. http://www.businessinsurance.org/the-truth-about-cancer-causing-cosmetics/
5. http://fitbeaut.blogspot.com/2012/10/paraben-free-cosmetics.html

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Fast Analysis of Cosmetic Allergens Using UltraPerformance Convergence Chromatography (UPC²) with MS Detection

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[2]Waters Corporation, Paris, France

APPLICATION BENEFITS

ACQUITY® UPC²® with MS detection offers:

- Efficient, cost effective analysis of cosmetic allergens, compared to standard methodology.
- Greater than six-fold increase in sample throughput, and greater than 95% reduction in toxic solvent usage than existing HPLC methods.
- The ability to handle traditional GC and LC amenable compounds in a single analysis using UPC.[2]

WATERS SOLUTIONS

ACQUITY UPC2 System

Xevo® TQD

ACQUITY UPC2 C_{18} HSS Column

MassLynx® MS Software

KEY WORDS

Allergens, cosmetics, perfume, Convergence Chromatography, supercritical fluid chromatography, SFC, personal care products, mass spectrometry

INTRODUCTION

Fragrances are complex combinations of natural and/or man-made substances that are added to many consumer products to give them a distinctive smell, impart a pleasant odor, or mask the inherent smell of some ingredients, but ultimately to enhance the experience of the product user. Fragrances create important olfactory benefits that are ubiquitous, tangible, and valued. Fragrances can be used to communicate complex ideas such as creating mood, signaling cleanliness, freshness, softness, alleviating stress, creating well-being, or to trigger allure and attraction.

In most types of cosmetics and skin care products, including perfumes, shampoos, conditioners, moisturizers, facial cosmetics, and deodorants, there are more than 5000 different fragrances present. Many people suffer from allergies, which are caused by an abnormal reaction of the body to a previously encountered allergen that can be introduced in a number of ways such as by inhalation, ingestion, injection, or skin contact. Allergies are often manifested by itchy eyes, a runny nose, wheezing, skin rashes (including dermatitis[1]), or diarrhea.

In the EU Cosmetic Regulations (1223/2009),[2] there are 'currently' 26 fragrance ingredients, 24 volatile chemicals, and two natural extracts (oak moss and tree moss), that are considered more likely to cause reactions in susceptible people. These 26 fragrance ingredients must be indicated in the list of ingredients of the final product, if the concentration exceeds 0.001% (10 mg/kg) in leave-on products, e.g. moisturizers, or 0.01% (100 mg/kg) in rinse-off products, e.g shampoos. Listing the regulated allergens on products can help identify the cause of an allergic reaction and also aids people to make informed choices about what they buy, particularly if they have a diagnosed allergy to a specific fragrance ingredient.

Current analytical methods used for the analysis of cosmetic allergens include Gas Chromatography Mass Spectrometry[3-5] (GC-MS), Headspace-GC-MS,[6] GC-GC/MS, Liquid Chromatography-UV (LC-UV),[7] and LC-MS,[8] which all have run times of approximately 30 to 40 minutes.

The current 24 regulated volatile cosmetic allergens contain compounds from different classes and different polarities (phenols, cyclic hydrocarbons, alcohols, carbonyl compounds, esters, and lactones). Many are small molecules with similar structures that often produce non-specific fragment ions for mass spectrometric detection.

There are many challenges that need to be addressed for any method used for allergen analysis. For example, the resolution achieved between analyte, isomer, and matrix components all need to be optimized, and the sensitivity of the method should be at least 1 ppm (greater preferred).

Convergence Chromatography (CC) is a separation technique that uses carbon dioxide as the primary mobile phase, with the option if required to use an additional co-solvent such as acetonitrile or methanol to give similar selectivity as normal phase LC.

This application note will consider how hyphenating Waters®UltraPerformance Convergence Chromatography™ (UPC^2) with MS detection can be used to achieve specificity, selectivity, and sensitivity for the analysis of fragrance allergens in perfume, cosmetics, and personal care products in a fast 7-minute run.

EXPERIMENTAL

Sample preparation

Cosmetic and personal care sample analysis

- 0.2 g sample was added to 2.5 mL water and 2.5 mL (methanol + 20 mM ammonium hydrogen carbonate).
- Mixture vortexed for 2 min (1600 rpm).
- Mixture further extracted in an ultrasonic bath for 30 min.
- Approximately 1-mL of extract centrifuged for 5 min (10,000 rpm).
- Centrifuged extract transferred to LC vials ready for analysis.

Perfume

100 µL sample + 900 µl (methanol + 20 mM ammonium hydrogen carbonate).

UPC^2 conditions

System:	ACQUITY UPC^2
Run time:	7.0 min
Column:	ACQUITY UPC^2 C_{18} HSS, 3.0 mm x 150 mm, 1.8 µm
Column temp.:	60 °C
CCM back pressure:	1500 psi
Sample temp.:	15 °C
Mobile phase A:	CO_2
Mobile phase B:	Methanol (0.1% formic acid)
Flow rate:	1.5 mL/min
Injection volume:	3 µL
Isocratic solvent manager solvent:	Methanol
Isocratic solvent manager flow rate:	0.4 mL/min
Vials:	Waters Amber Glass 12 x 32 mm Screw Neck, 2 mL, part no. 186007200C

Mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	1.5	99.5	0.5	–
2	4.50	1.5	85.4	14.6	6
3	4.60	1.5	80.0	20.0	6
4	5.00	1.5	80.0	20.0	6
5	5.05	1.5	99.5	0.5	6
6	7.00	1.5	99.5	0.5	6

Table 1. ACQUITY UPC^2 mobile phase gradient.

MS conditions

MS system:	Xevo TQD
Ionization mode:	APCI (+ and -)
Corona voltage:	10 µA
Source temp.:	150 °C
APCI probe temp.:	600 °C
Desolvation gas:	1000 L/hr
Cone gas:	15 L/hr
Acquisition:	Multiple Reaction Monitoring (MRM)

The MS conditions were optimized for the analysis of 24 currently regulated cosmetic allergens. Six additional compounds were also analyzed, considering cosmetic allergens that could potentially be added during future regulation changes, and two compounds that are potential carcinogens (methyl eugenol and 4-allyl anisole). CAS numbers, empirical formulas, and structures are detailed in Table 2 and Table 3 respectively. The established MRM method (Table 4) utilizes fast polarity switching available on the Xevo TQD, which enables the analysis of positive and negative allergens within the same analytical analysis.

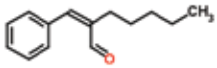
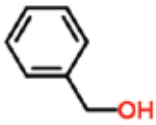
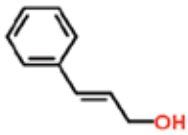
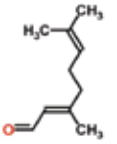
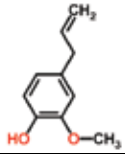
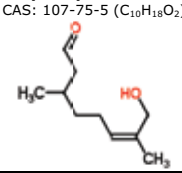
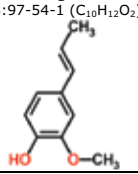
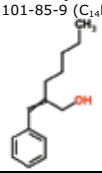
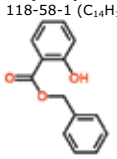
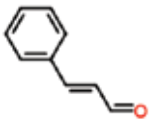
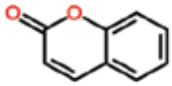
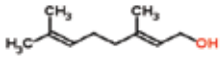
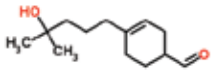
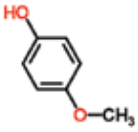
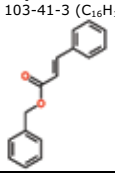
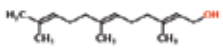
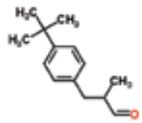
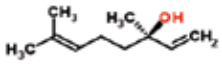
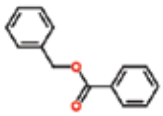
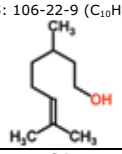
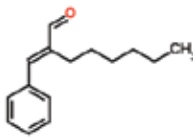
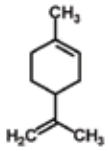
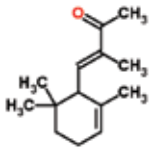
Regulated Allergens[2]			
1. **Amyl Cinnamaldehyde** CAS: 122-40-7 ($C_{14}H_{18}O$) 	**2.** **Benzyl alcohol** CAS: 100-51-6 (C_7H_8O) 	**3.** **Cinnamyl alcohol** CAS: 104-54-1 ($C_9H_{10}O$) 	**4.** **Citral** CAS: 5392-40-5 ($C_{10}H_{16}O$) 
5. **Eugenol** CAS: 97-54-0 ($C_{10}H_{12}O_2$) 	**6.** **Hydroxy-citronellal** CAS: 107-75-5 ($C_{10}H_{18}O_2$) 	**7.** **Isoeugenol** CAS:97-54-1 ($C_{10}H_{12}O_2$) 	**8.** **Amyl cinnamyl alcohol** CAS: 101-85-9 ($C_{14}H_{20}O$) 
9. **Benzyl salycilate** CAS: 118-58-1 ($C_{14}H_{12}O_3$) 	**10.** **Cinnamaldehyde** CAS: 104-55-2 (C_9H_8O) 	**11.** **Coumarin** CAS: 94-64-5 ($C_9H_6O_2$) 	**12.** **Geraniol** CAS: 106-24-1 ($C_{10}H_{18}O$) 
13. **Lyral** CAS: 31906-04-4 ($C_{13}H_{22}O_2$) 	**14.** **Anisyl alcohol** CAS: 105-13-5 ($C_7H_8O_2$) 	**15.** **Benzyl cinnamate** CAS: 103-41-3 ($C_{16}H_{14}O_2$) 	**16.** **Farnesol** CAS: 4602-84-0 ($C_{15}H_{26}O$) 
17. **Lilial** CAS: 80-54-6 ($C_{14}H_{20}O$) 	**18.** **Linalool** CAS: 78-70-6 ($C_{10}H_{18}O$) 	**19.** **Benzyl benzoate** CAS: 120-51-4 ($C_{14}H_{12}O_2$) 	**20.** **Citronellol** CAS: 106-22-9 ($C_{10}H_{20}O$) 
21. **Hexyl cinnamaldehyde** CAS: 101-86-0 ($C_{15}H_{20}O$) 	**22.** **Limonene** CAS: 5989-27-5 ($C_{10}H_{16}$) 	**23.** **Methyl heptine carbonate** CAS: 111-12-6 ($C_9H_{14}O_2$) 	**24.** **Alpha isomethyl ionone** CAS: 127-51-5 ($C_{14}H_{22}O$) 

Table 2. Cosmetic allergens considered, as regulated under current EU Cosmetic Regulations 1223/2009,[2] associated CAS numbers, empirical formulas, and structures.

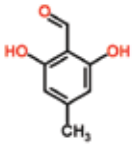
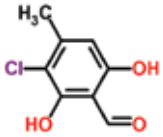
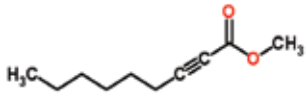
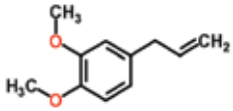
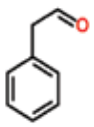
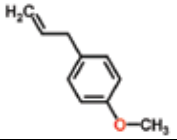
Additional compounds considered		
25. **Atranol** CAS: 526-37-4 ($C_8H_8O_3$) 	**26.** **Chloratranol** CAS: 57074-21-2 ($C_8H_7ClO_3$) 	**27.** **Methyl-2-nonynoate** CAS: 111-80-8 ($C_{10}H_{16}O_2$) 
28. **Methyl eugenol** CAS: 93-15-2 ($C_{11}H_{14}O_2$) 	**29.** **Phenylacetaldehyde** CAS: 122-78-1 (C_8H_8O) 	**30.** **4-Allyl anisole** CAS: 140-67-0 ($C_{10}H_{12}O$) 

Table 3. Additional compounds considered, associated CAS numbers, empirical formulas, and structures.

No	Chemical substance	Retention time (min) #isomers	APCI (+/-)	Cone voltage (V)	Transition	Collision energy
1	Amyl cinnamaldehyde	1.84	+	30	203.0>129.0*	18
					203.0>147.0	16
2	Benzyl alcohol	1.86	+	8	155.0>91.0*	8
					155.0>123.0	4
3	Cinnamyl alcohol	2.78	+	25	133.0>185.0*	18
4	Citral	1.58	+	15	153.0>69.0*	6
					153.0>95.0	15
5	Eugenol	1.68	+	20	165.1>124.0	20
					165.1>137.1*	12
6	Hydroxy-citronellal	3.37	+	18	171.0>111.0	15
					171.0>153.0*	10
7	Isoeugenol	1.90	+	25	165.1>105.0	20
					165.1>133.0*	20
8	Amyl cinnamyl alcohol	2.84	+	25	187.0>117.0*	20
					187.0>131.0	16
9	Benzyl salycilate	1.86	+	15	229.0>91.0*	12
					229.0>151.0	12
10	Cinnamaldehyde	1.75	+	25	133.0>55.0*	18
					133.0>115.0	14
11	Coumarine	2.52	+	40	147.0>91.0	28
					147.0>103.0*	23
12	Geraniol	1.59	+	20	137.0>81.0*	14
					137.0 >95.0	16
13	Lyral	3.24	+	20	193.0>111.0	18
					193.0>175.0*	12
14	Anisyl alcohol	2.79	+	40	121.0>77.0*	25
					121.0>78.0	25
15	Benzyl cinnamate	2.31	+	25	221.0>105.0	6
					221.0>193.0*	8
16	Farnesol	2.61/2.76/2.83#	+	25	205.1>109.0	20
					205.1>121.0*	20
17	Lilial	2.31	+	10	221.2>90.9*	30
18	Linalool	2.23	+	20	137.0>81.0*	20
					137.0>95.0	20
19	Benzyl benzoate	1.87	+	8	213.0>91.0*	8
20	Citronellol	2.19	+	18	157.1>57.0	10
					157.1>83.0*	10
21	Hexyl cinnamaldehyde	1.94	+	30	217.4>129*	20
					217.4>147	14
22	Limonene	0.67	+	20	137.0>81.0*	14
					137.0>95.0	16
23	Methyl heptine carbonate	0.72	+	30	155.0>67.0*	24
					155.0>123.0	15
24	Alpha isomethyl ionone	1.65	+	20	207.2>111.1*	20
					207.2>123.1	20
25	Atranol	4.57	-	18	151.0>78.94*	20
					151.0>123.09	20
26	Chloratranol	2.90	-	18	185.0>121.17*	20
					185.0>156.99	20
27	Methyl-2-nonynoate	1.53	+	34	153.0>42.9	22
					153.0>97.0*	16
28	Methyl eugenol	1.78	+	25	179.0>138*	16
					179.0>164	14
29	Phenylacetaldehyde	0.70	+	2	121.0>56.9	4
					121.0>89.0*	10
30	4-Allyl anisole	2.52	+	30	146.9>76.9	28
					146.9>90.9*	32

Table 4. Expected retention times, ionization mode, cone voltages, MRM transitions, and associated collision energy values for 24 regulated cosmetic allergens and six additional compounds.

Instrument control, data acquisition, and results processing

MassLynx Software was used to control the ACQUITY UPC^2 and the Xevo TQD, and also for data acquisition. Data quantitation was achieved using the TargetLynx™ Application Manager.

RESULTS AND DISCUSSION

The analysis of the 24 regulated and 6 additional compounds was achieved using the Xevo TQD in MRM mode with APCI ionization (+/-), coupled to an ACQUITY UPC^2 System.

Optimum MRM and UPC^2 conditions were developed with the elution of all compounds within a 7-minute run.

Mixed calibration standards, 0.25 to 25 ppm, were prepared and analyzed. An example calibration curve generated for cinamyl alcohol, shown in Figure 1, with an r^2 value of 0.9999. The MRM chromatograms for each compound are shown in Figure 2.

The developed 7-minute UPC^2 method, is more than six times faster than existing HPLC and GC methods, with an excess of 95% less solvent usage than existing HPLC methods.

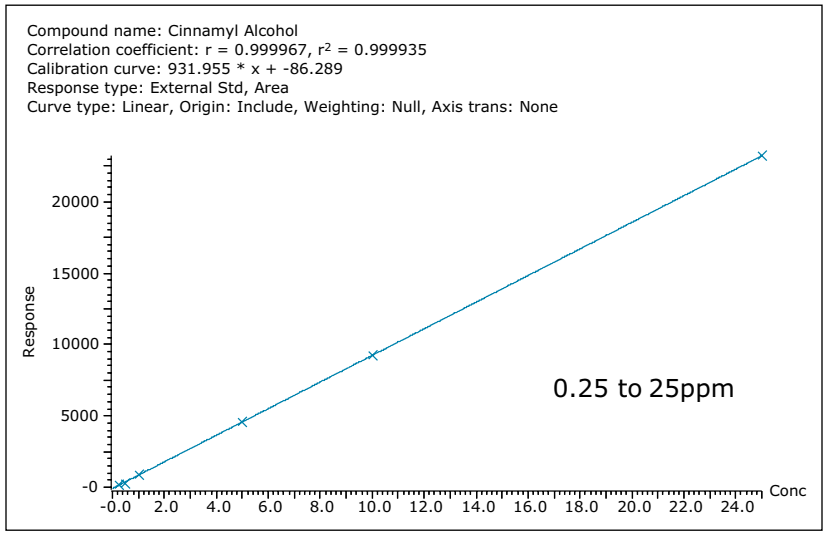


Figure 1. TargetLynx Quantify results browser showing the calibration curve for cinnamyl alcohol.

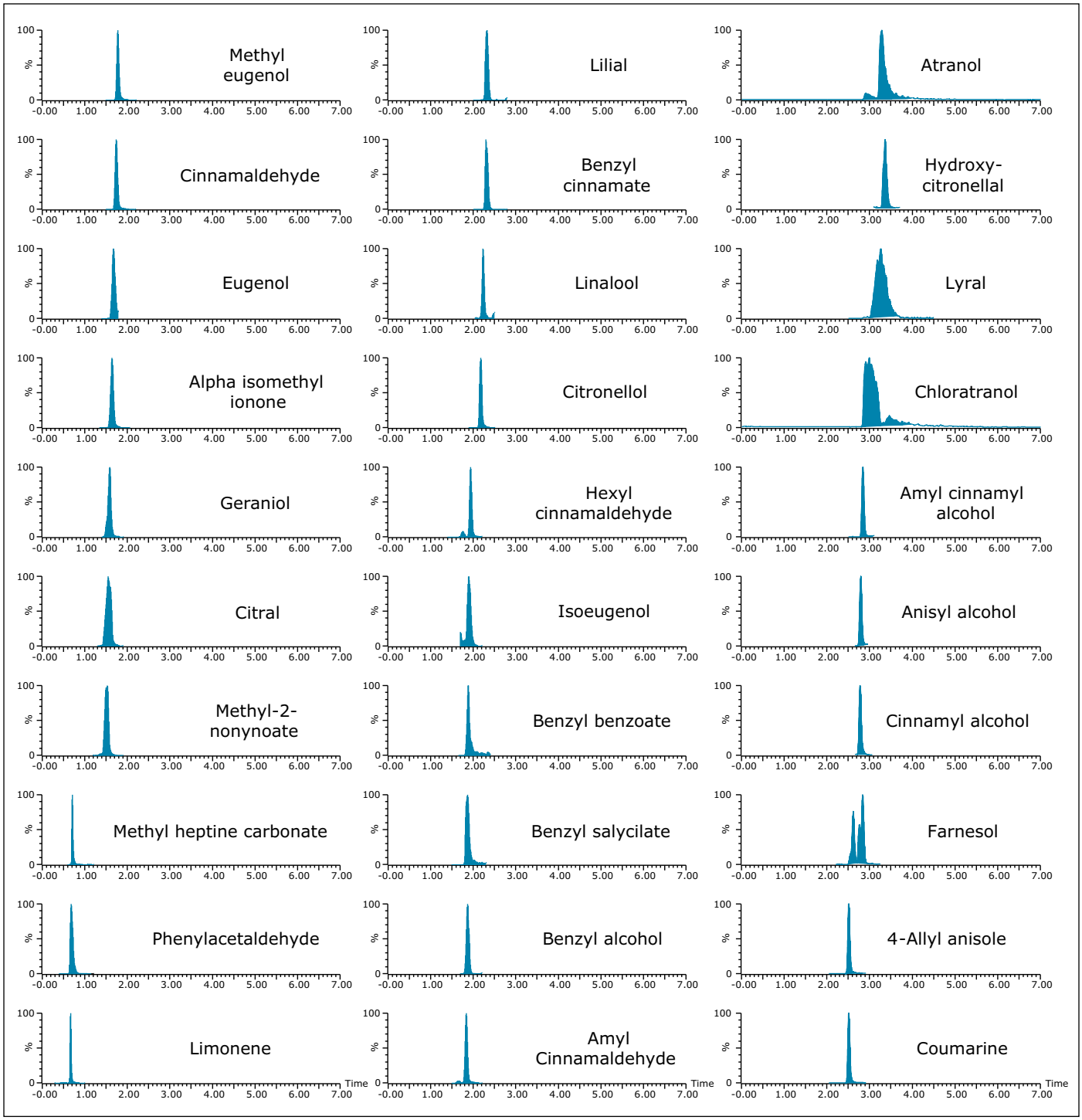


Figure 2. MRM chromatograms for 24 regulated cosmetic allergens and six additional compounds in 10 ppm calibration standards (1 ppm for chloratranol and atranol).

Shampoo and perfume analysis

The MRM mass detection method (Table 4) was used after appropriate sample preparation for the analysis of the 24 regulated and four additional compounds in shampoo and perfume samples.

Perfume samples were fortified at 10 mg/kg (0.001%) with 24 cosmetic allergens, and four additional compounds. They were then prepared for analysis as detailed in the Experimental section. Example MRM chromatograms achieved for fortified perfume are shown in Figure 3.

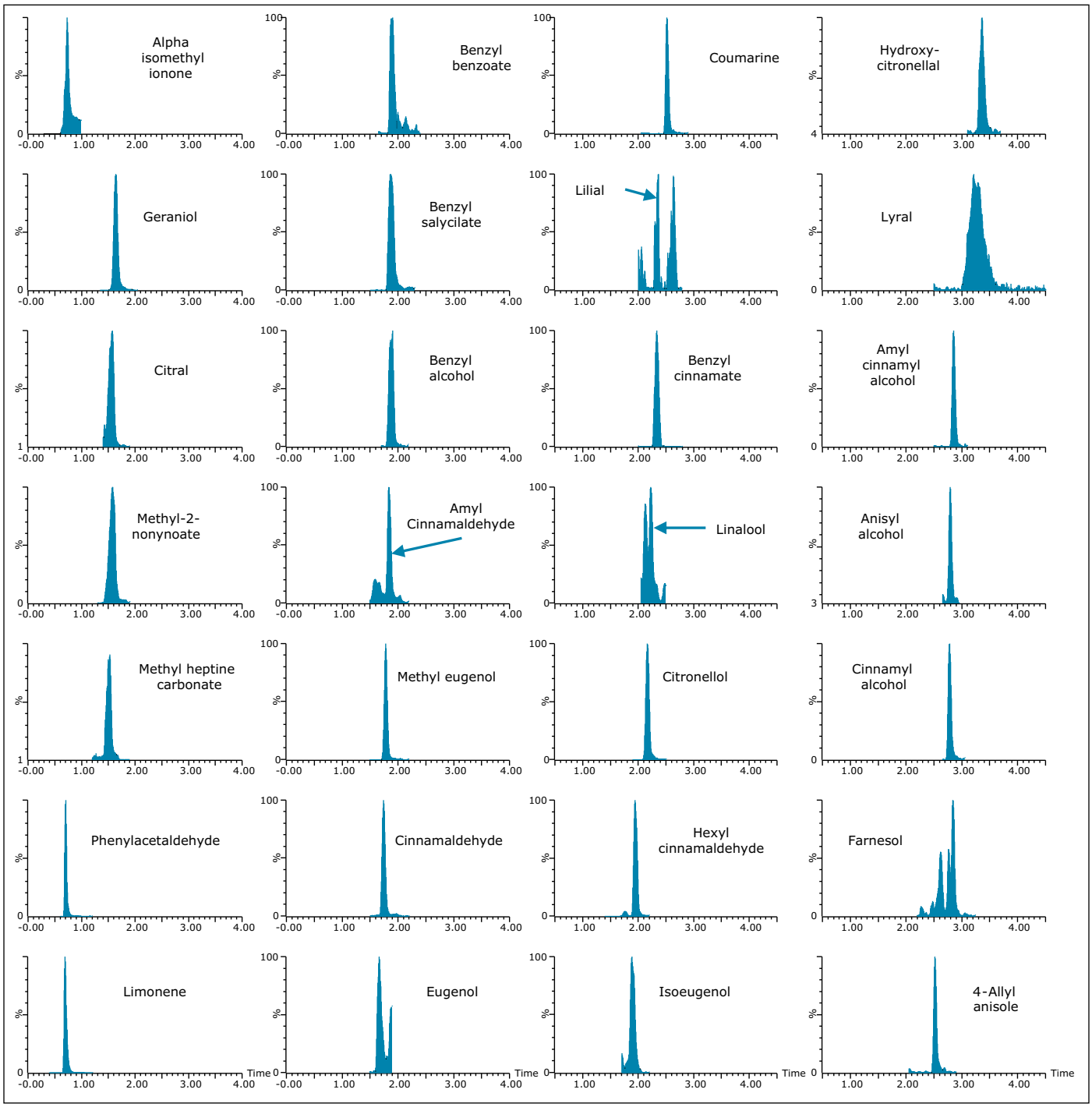


Figure 3. MRM chromatograms for 24 cosmetic allergens and four additional compounds in perfume, fortified at 10 mg/kg (0.001%).

Shampoo samples were fortified at 100 mg/kg (0.01%) with 24 cosmetic allergens and 4 additional compounds, then prepared for analysis as detailed in the Experimental section. Example MRM chromatograms achieved for fortified shampoo are shown in Figure 4.

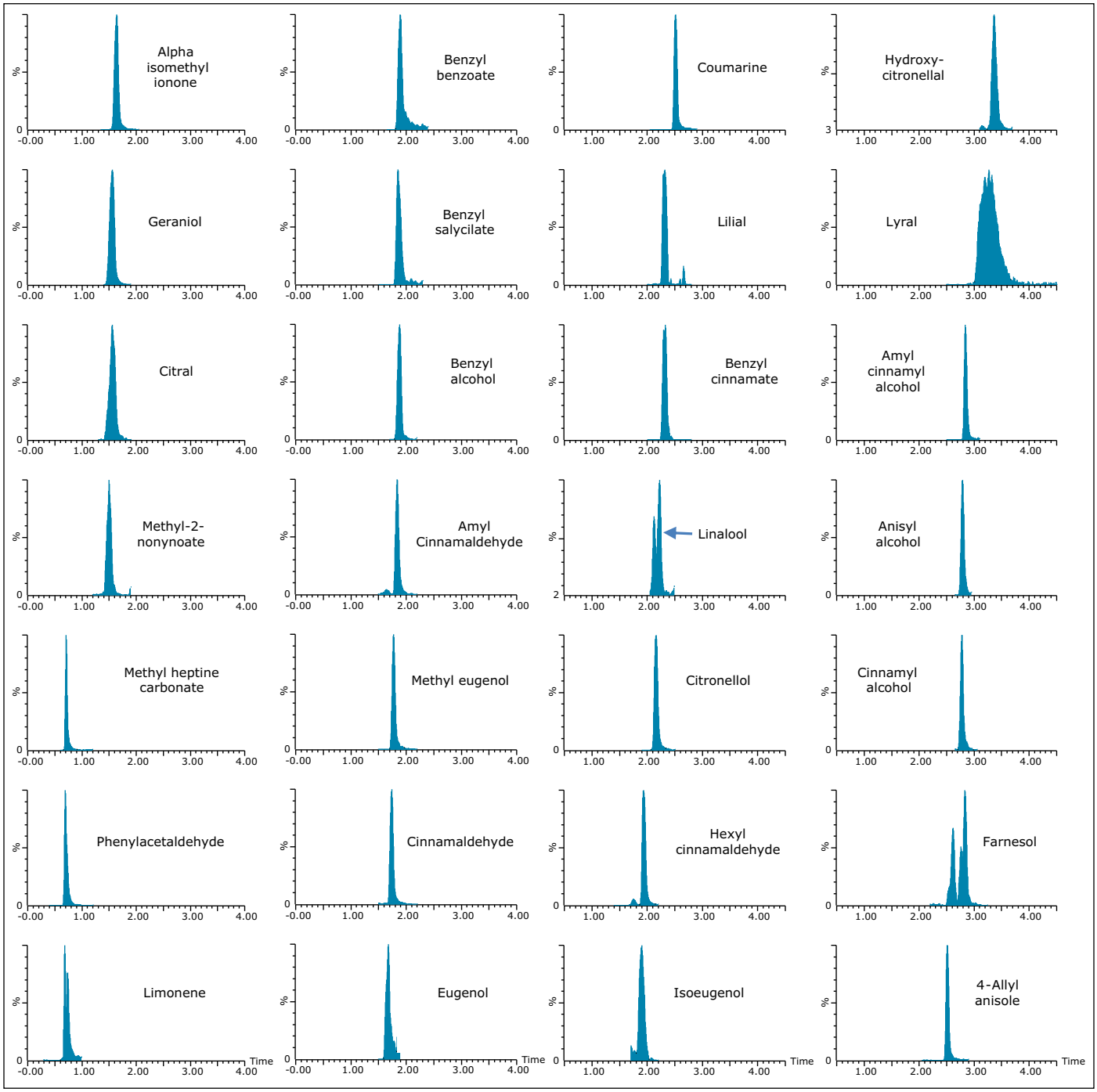


Figure 4. MRM chromatograms for 24 cosmetic allergens and 4 additional compounds in shampoo fortified at 100 mg/kg (0.01%).

Various cosmetic allergens compounds are isomeric, for example Farnesol where potentially four isomeric forms can be produced (Figure 5). For the example of farnesol, normally trans,trans-farnesol is the major isomer, with trans,cis-farnesol and cis,trans-farnesol being the minor forms, leaving cis,cis-farnesol which is rarely seen. This is demonstrated by the MRM chromatograms (Figure 6) for farnesol in a shampoo sample fortified at 10 mg/Kg (one tenth of the regulated limit of 0.01%), and the nearest equivalent standard (0.5 ppm), which illustrated several isomeric farnesol peaks. For comparison, a blank shampoo sample MRM chromatogram for farnesol is also shown in Figure 6.

Additional benefits of using ACQUITY UPC^2 coupled to the Xevo TQD over previous methodology include improved selectivity and sensitivity for the analysis of cosmetic allergens. The established method achieves resolution between analytes, isomers, and matrix. Additionally, the attained sensitivity is four times less than required (0.25 ppm).

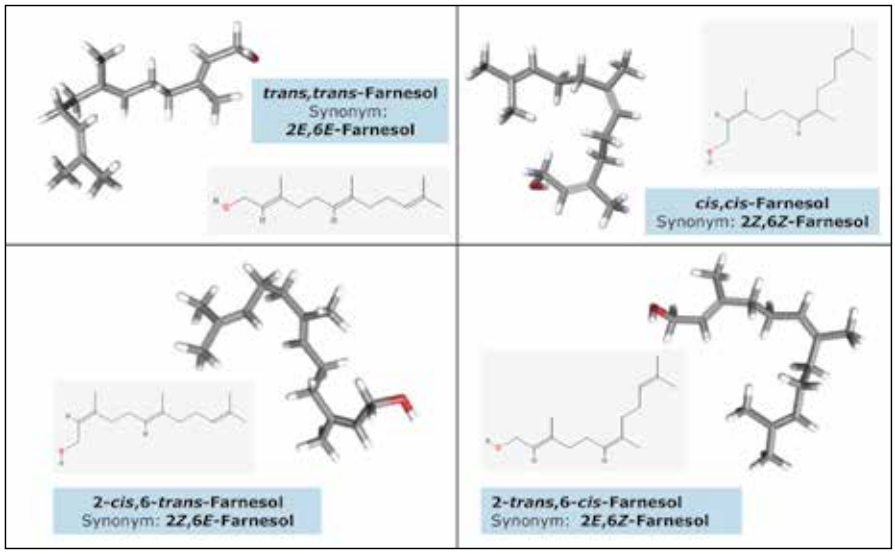


Figure 5. Four isomers of farnesol.

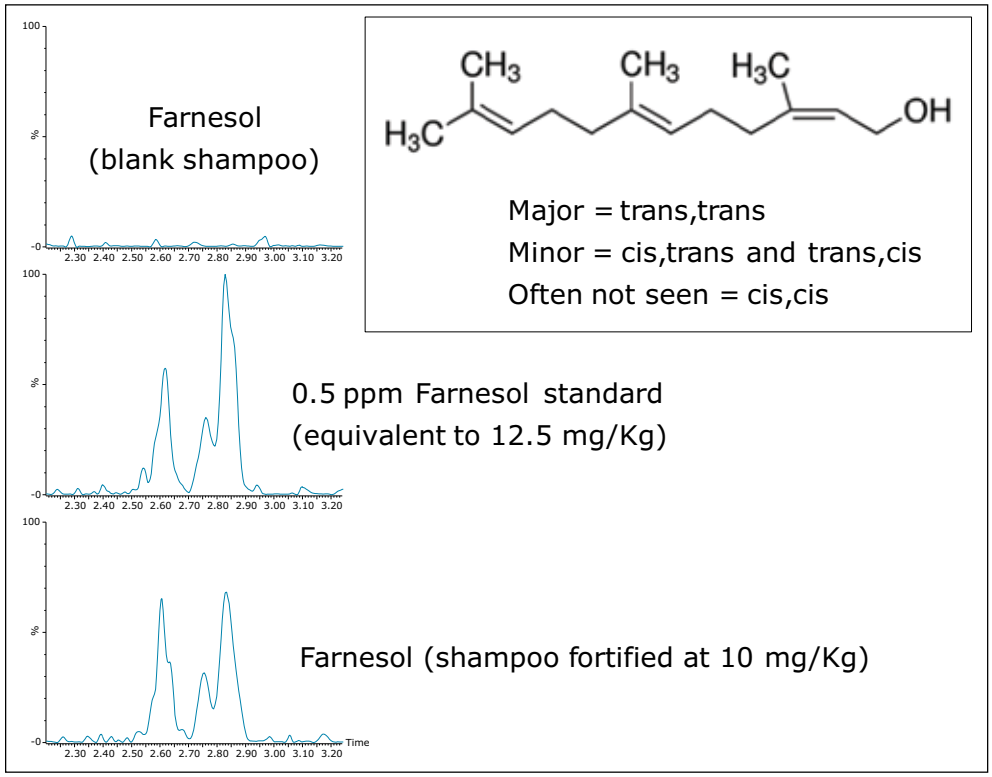


Figure 6. MRM chromatograms for shampoo fortified at 10 mg/Kg (one-tenth of the regulated limit of 0.01%), the nearest equivalent standard (10 mg/Kg), and a blank shampoo sample.

CONCLUSIONS

- Separation by UPC^2 is an ideal alternative to both HPLC and GC analysis.
- Ability to run LC and GC amenable compounds in a single analysis.
- Fast 7-minute analysis of the 24 regulated cosmetic allergens, 4 non-regulated cosmetic allergens, and 2 potential carcinogenic compounds containing:
  - different classes of compounds;
  - different polarities.
- UPC^2 with MS detection offers an orthogonal technique, which enables greater selectivity and specificity compared to either HPLC or GC analysis alone.
- The developed 7-minute UPC^2 method is more than six times faster than existing HPLC and GC methods, with 95% less solvent usage than existing HPLC methods.

Acknowledgements

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References

1. Larson W, Nakayama H, Fischer T, et al. Fragrance contact dermatitis: a worldwide multicenter investigation (Part II). *Contact Dermatitis*. 44(6): 344–346, June 2001.
2. The European Parliament and the Council of the European Union. Regulations (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. *Official Journal of the European Union*. L 342/59: 59-209, 22nd Dec 2009. [cited 2015 January 15]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF
3. Debonneville C, Chaintreau A. Quantitation of suspected allergens in fragrances: evaluation of comprehensive GC-conventional MS. *J Chromatogr A*. 1027: 109–15, 2004.
4. Rastogi S C. Analysis of Fragrances in Cosmetics by Gas Chromatography-Mass Spectrometry. *J High Resolution Chrom*. 18: 653–658, 1995.
5. López-Nogueroles M, Chisvert A, Salvador A. Determination of atranol and chloratranol in perfumes using simultaneous derivatization and dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. *Analytica Chimica Acta*. 826: 28–34, 2014.
6. Desmedt B, Canfyn M, Pype M, et al. HS-GC-MS method for the analysis of fragrance allergens in complex cosmetic matrices. *Talanta*. 131: 444–451, 2015.
7. Villa C, Gambaro R, Mariani E, Dorato S. High-performance liquid chromatographic method for the simultaneous determination of 24 fragrance allergens to study scented products. *J Pharmaceutical and Biomedical Analysis*. 44: 755–762, 2007.
8. Rudback J, Islam N, Nilsson U, et al. A sensitive method for determination of allergenic fragrance terpene hydroperoxides using liquid chromatography coupled with tandem mass spectrometry. *J Sep Sci.* 36:1370–1378, 2013.

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Non-Targeted Screening Analysis of Packaging Extracts Using the UNIFI Scientific Information System

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APPLICATION BENEFITS

- Simple LC-MS methodology leverages high-resolution mass spectrometry that can be adopted for cosmetics, food, and pharmaceutical packaging extractable applications.
- Streamlines the structural elucidation process for packaging extracts by utilizing MS^E data of accurate mass precursor and fragment ion information on a single software platform.
- Rapidly evaluate information for an unknown component (*m/z*) by ranking the possible elemental compositions and performing database searches for likely structures ranked based on fragmentation matching.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

UNIFI® Scientific Information System

Xevo® G2-XS QTof Mass Spectrometer

CORTECS® C_{18} Column

KEY WORDS

Extractables, leachables, packaging, cosmetics, screening, elucidation, accurate mass, QTof, non-targeted analysis, informatics

INTRODUCTION

Characterization of packaging in various industries has become more important due to ever-increasing global regulations. The first regulations for plastics used in food packaging and contact materials were established in 1982 in Europe,[1] which have been expanded in recent years.[2] In the pharmaceutical field the need for extractables testing was recognized in the 1990s.[3] Manufacturers are required to evaluate packaging for the possible migration of additives and ingredients into the final product because of the potential impact extractables and leachables can have on patients' health.[4,5] Extractables in the pharmaceutical industry are defined as compounds that can be extracted from packaging materials or devices under controlled experimental conditions. Leachables, a subset of extractables, are compounds that actually migrate into the final product during expected shelf or contact time. The latest addition to the industries that require testing of packaging is the cosmetics industry. The most recent regulations for the cosmetics industry in Europe (EU Regulation 1223/2009) Annex 1 states that "impurities, traces, information about the packaging material must be determined".[6] For the cosmetics industry the impact from leachables would depend on the route of application. For example, it would be less critical for cosmetic products that are applied to the skin such as body creams than it would for products that can be ingested or absorbed through the eyes, such as lipstick or mascara.

The initial step for characterizing extractables from packaging involves targeted screening, *i.e.*, testing the extracts for known compounds. This is a well-established process that can be performed using various analytical techniques ranging from GC-FID-MS to LC-UV/MS. However, the final packaging may have impurities present from the starting materials and additional degradants such as those formed during the molding process. The first step in ensuring that these compounds do not pose any toxicological risks to the consumer is to identify the extractables, or at least their structural class. The structural elucidation of unknowns is typically a very complex and time-consuming process that requires the analyst to have a higher level of expertise. Waters® UNIFI Scientific Information System utilizes accurate mass and fragment information to simplify data review and facilitate the decision-making process. It allows analysts to evaluate complex data in a more efficient way and quickly make decisions about the possible identity of an unknown compound.

EXPERIMENTAL

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class
Separation mode:	Gradient
Column:	CORTECS UPLC C_{18} 90Å, 1.6 µm, 2.1 mm x 100 mm
Column temp.:	40 °C
Injection volume:	5 µL
Flow rate:	0.5 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient:	60% B held for 30s, increased to 99% over 2.5 min, held at 99% for 5 min, then re-equilibrated back to 60%

MS conditions

MS system:	Xevo G2-XS QTof
Ionization mode:	ESI +
Capillary voltage:	3.0 kV
Desolvation temp.:	450 °C
Source temp.:	150 °C
Cone voltage:	25 V
Collision ramp:	10 to 40 eV
MS scan range:	50 to 1200 *m/z*

Data acquisition and processing

UNIFI Software was used for acquisition and data processing.

Sample preparation

Mascara packaging made of polypropylene, lipstick packaging and tonal cream packaging made of polyethylene were chosen as samples. The cosmetics products were removed from the packaging, which was subsequently cut into 1x1 cm pieces. Sample extracts were prepared in isopropanol (IPA) by extracting ~2 g in 5 mL of IPA by sonication in glass scintillation vials for 6 hours.

RESULTS AND DISCUSSION

Typically, screening experiments for packaging extracts are performed using generic gradient LC-MS methods. As it is not known what kind of chromatographic profile the extract might have, the screening methods are not optimized for each individual packaging material at this initial stage in R&D. If the chromatogram only has one or two peaks, it is easy for analysts to decide where to start their investigation. However, if the extract has a multiple chromatographic peaks that are not completely resolved, or if several groups of samples must be compared, the analyst needs to determine which compounds are unique to the extract and are not present in the extraction blank (Figure 1). Furthermore, less intensively ionized compounds or trace-level compounds of toxicological concern may not be visible in the total ion current (TIC) chromatogram, or even in the base peak intensity (BPI) chromatogram.

Binary compare

In cases where only two samples must be compared, for example a blank extract (reference) and a sample (unknown), UNIFI Software's binary comparison feature allows the analyst to directly compare the chromatographic and spectral results of an analyte sample with those of a reference sample. Masses (*m/z*) in the reference and unknown spectra are considered to be the same component if they are within the user-specified mass, retention time, and intensity difference tolerance. The comparison can be presented graphically as a mirror image of BPI or TIC chromatograms, or as a table of Candidate Masses (Figure 2). The candidates are accurate mass and retention time pairs which have common peak features in the raw data. They are grouped according to retention time alignment and isotope spacing.

UNIFI shows a comparison between the mass spectrum of the compound in the unknown sample with the reference sample, and displays any differences. Figure 2 shows the comparison between an IPA blank extract "Reference sample" and lipstick packaging extract "Unknown sample" with the column "Match type" highlighting if the candidate is present in only the unknown sample, the reference sample, or both - the corresponding match types would be Unknown Unique, Reference Unique or Common. In this case, the most interesting candidates for further evaluation would be those that are not present in the extraction blank- Unknown Unique.

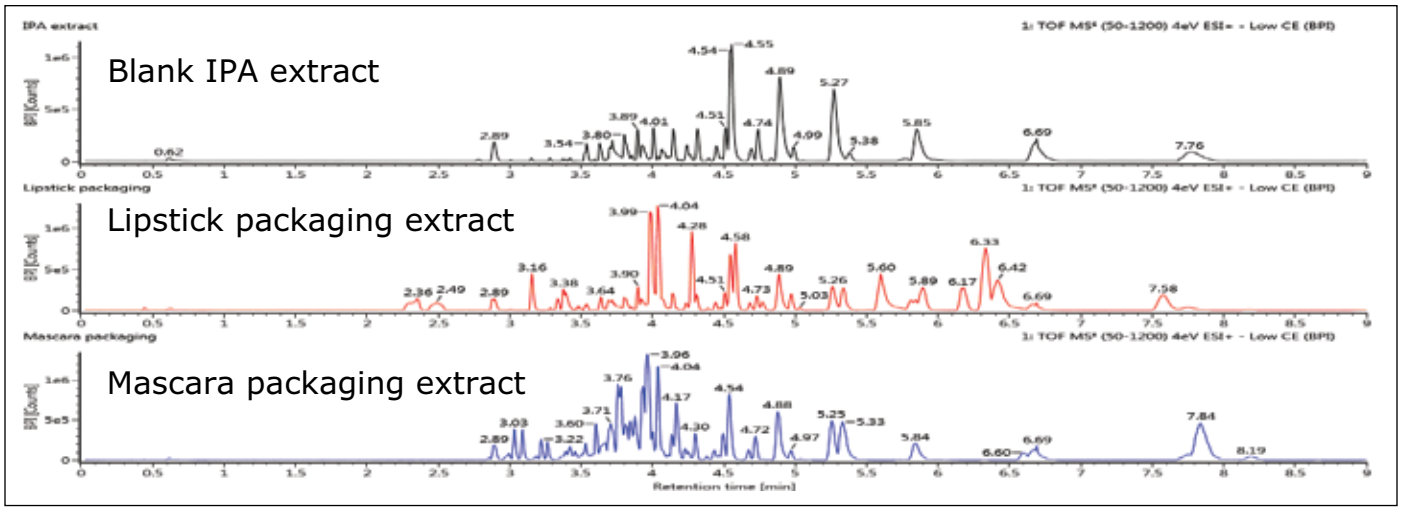

Figure 1. Mass chromatograms for packaging extracts and a blank extract.

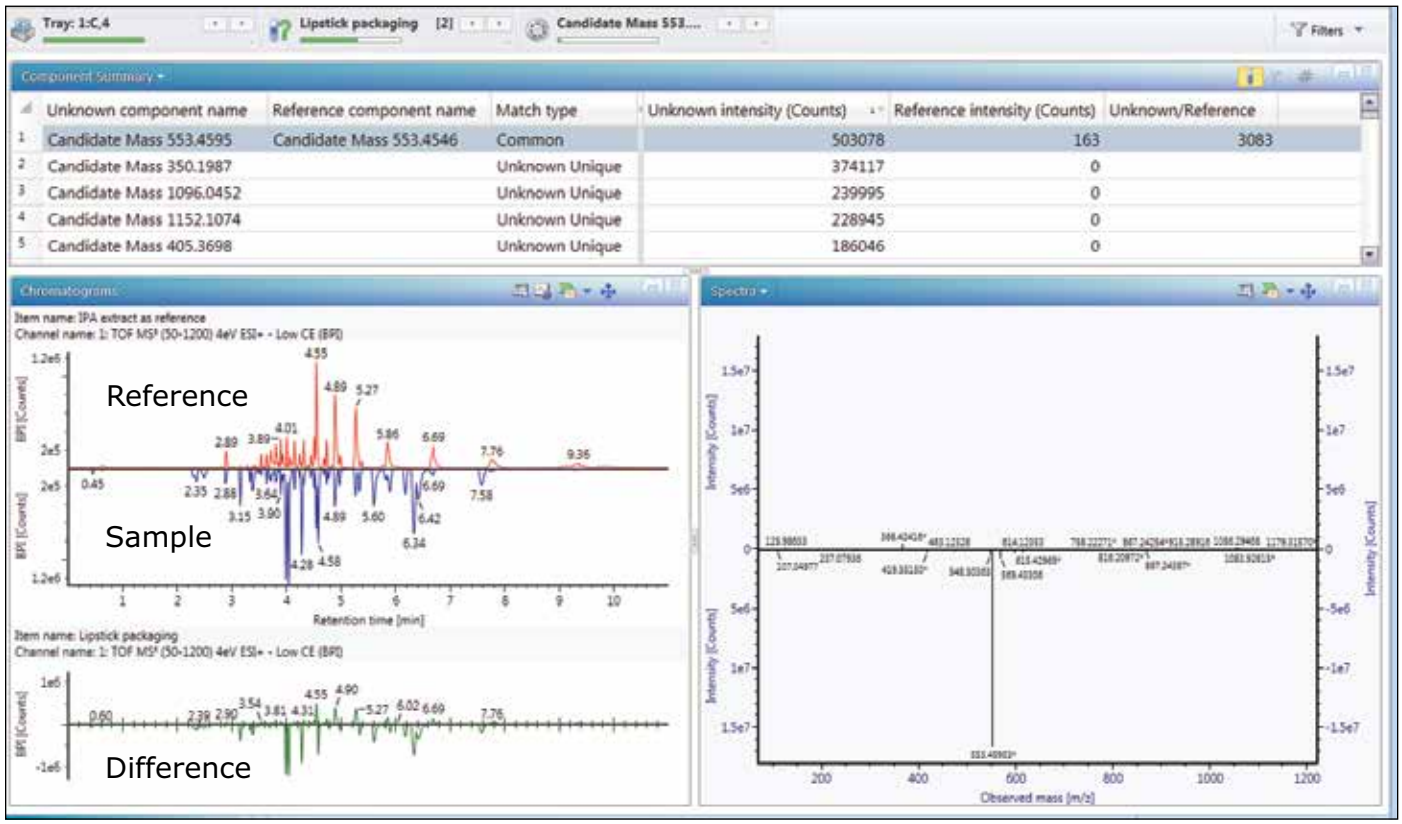

Figure 2. Binary compare results window for the IPA reference sample extract and lipstick packaging sample. The red trace shows the BPI chromatogram of the reference sample (IPA blank extract); the blue trace shows the BPI chromatogram of the lipstick packaging extract; and the green trace shows the difference between the samples.

Due to increases in instrument sensitivity and the ubiquitous presence of many extractables in LC-MS solvents, extraction vessels, plastic pipette tips, *etc.*, it is often difficult to obtain a clean blank. It is useful to evaluate the compounds where the candidate intensity in the unknown sample is much higher than in the reference sample. The column labeled Unknown/Reference (Figure 2) shows a ratio for common components, allowing users to quickly identify common extractables that may be persistent, but have a fold change that is significant. For candidate mass *m/z* 553.4595 the response ratio is over 3000 which indicates potential presence of the candidate in the extraction blank or a carryover.

High resolution mass spectrometry provides very comprehensive, high-quality information, but interpreting the data sets manually can be challenging. Therefore data processing software is of utmost importance for managing and reviewing data in an more efficient way. UNIFI Software allows users to set up their workflow in order to facilitate visualization of their data in the most productive way, and only display data that is relevant – all with a single click. The processed data can then be filtered using criteria defined by the user. In this case, to make the information in the table easier to manage the data was filtered based on specifications that showed Unknown Unique candidate masses with an intensity over 10,000 counts and Common candidate masses with a response ratio of Unknown/Reference of at least 300.

Once the data has been organized in a way that is most appropriate for the analyst, the next step is to proceed to elucidation of the candidates of interest (most intense for example) by utilizing the accurate mass information and high-collision energy fragment information.

Multivariate analysis (MVA)

Binary compare is useful for comparing two samples, but when multiple samples or sample groups need to be compared, the use of multivariate statistical analysis tools such as principal component analysis (PCA) facilitate the identification of differences between samples or groups. UNIFI can generate marker matrices based upon user-defined criteria which can then be automatically transferred to EZInfo 3.0.3 for MVA. PCA is a statistical tool that reduces a large set of multivariate data into uncorrelated variables called principal components. If additional discrimination among the investigated sample groups is required, the differences can be emphasized by using a Projection to Latent Structures Discriminant Analysis (PLS-DA) model (Figure 3). PLS-DA creates models of the quantitative relationships between the variables X (predictors) and Y (responses) for all sample groups. However, in these plots, each sample is presented by a single point, which does not allow individual markers contributing to the differences between the groups to be observed.

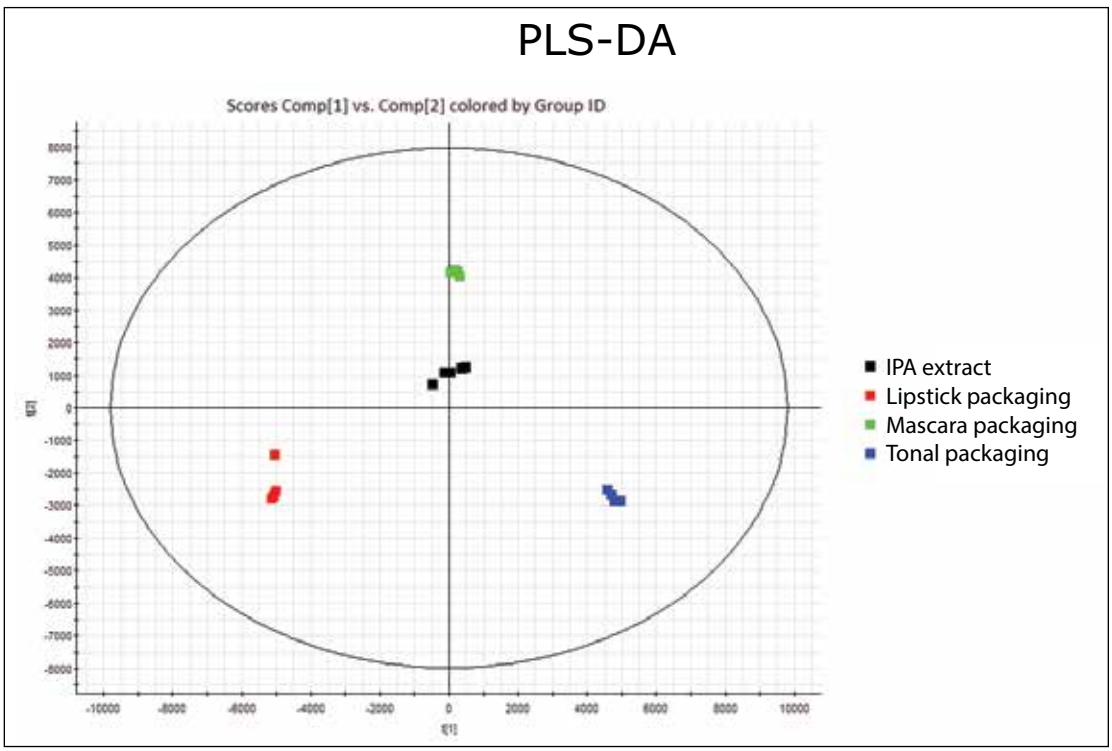

Figure 3. PLS-DA model for all of the packaging sample groups.

In order to investigate group differences down to individual markers, a loadings plot can be used. The loadings plot displays how the X variables correlate to each other, with points further away from the center being the most dissimilar between the sample groups (Figure 4). The data points in these plots are called Accurate Mass/Retention Time (AMRT) pairs. The quadrants in the loadings plot correspond to the PLS-DA model, thus the AMRTs in the lower left quadrant represent the unique markers in the lipstick packaging. Markers selected in red contribute most to the difference between the lipstick packaging and all the other packaging samples.

The differences between the groups can come from analytes that are not present in one of the groups, or from analytes with the greatest change in intensity (concentration) between the groups.

The individual markers that represented the biggest differences between the lipstick packaging and the rest of the group were selected (highlighted in red in Figure 4) and transferred back into UNIFI's Discovery tool for elucidation. When transferring selected markers from the loadings plot, labels can be added to make the data easier to sort and keep track of markers from different sample groups (Figure 5). When an individual marker is selected from the Marker Matrix table, a trend plot is displayed which allows users to quickly evaluate its presence in the other samples or injections.

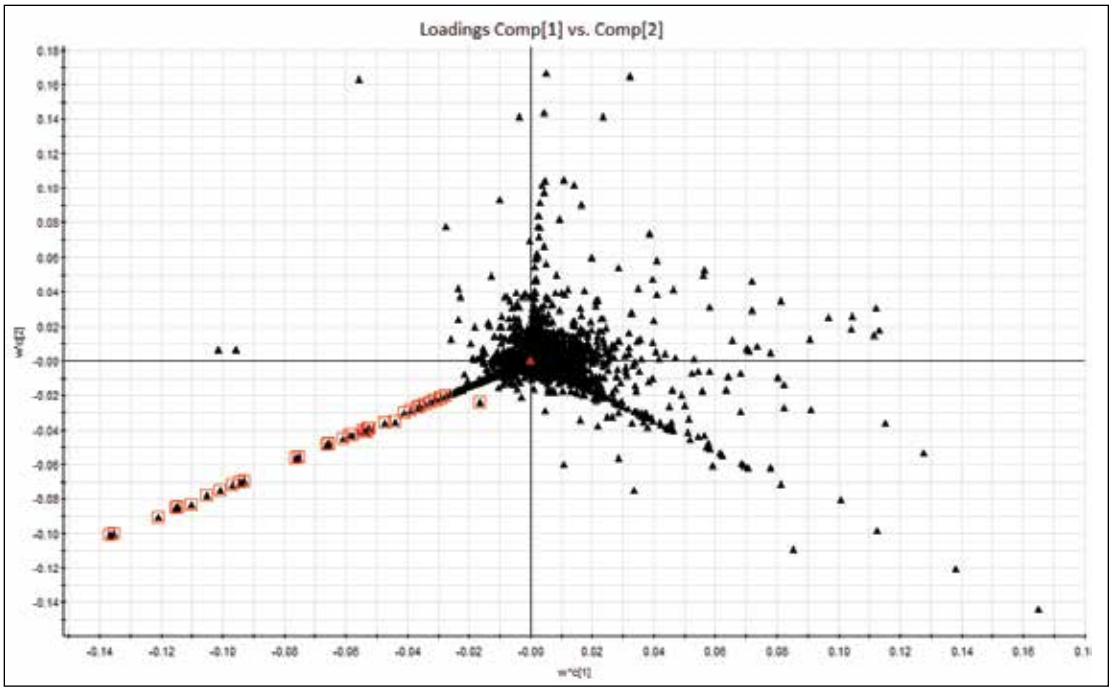

Figure 4. Loadings plot for all of the packaging samples.

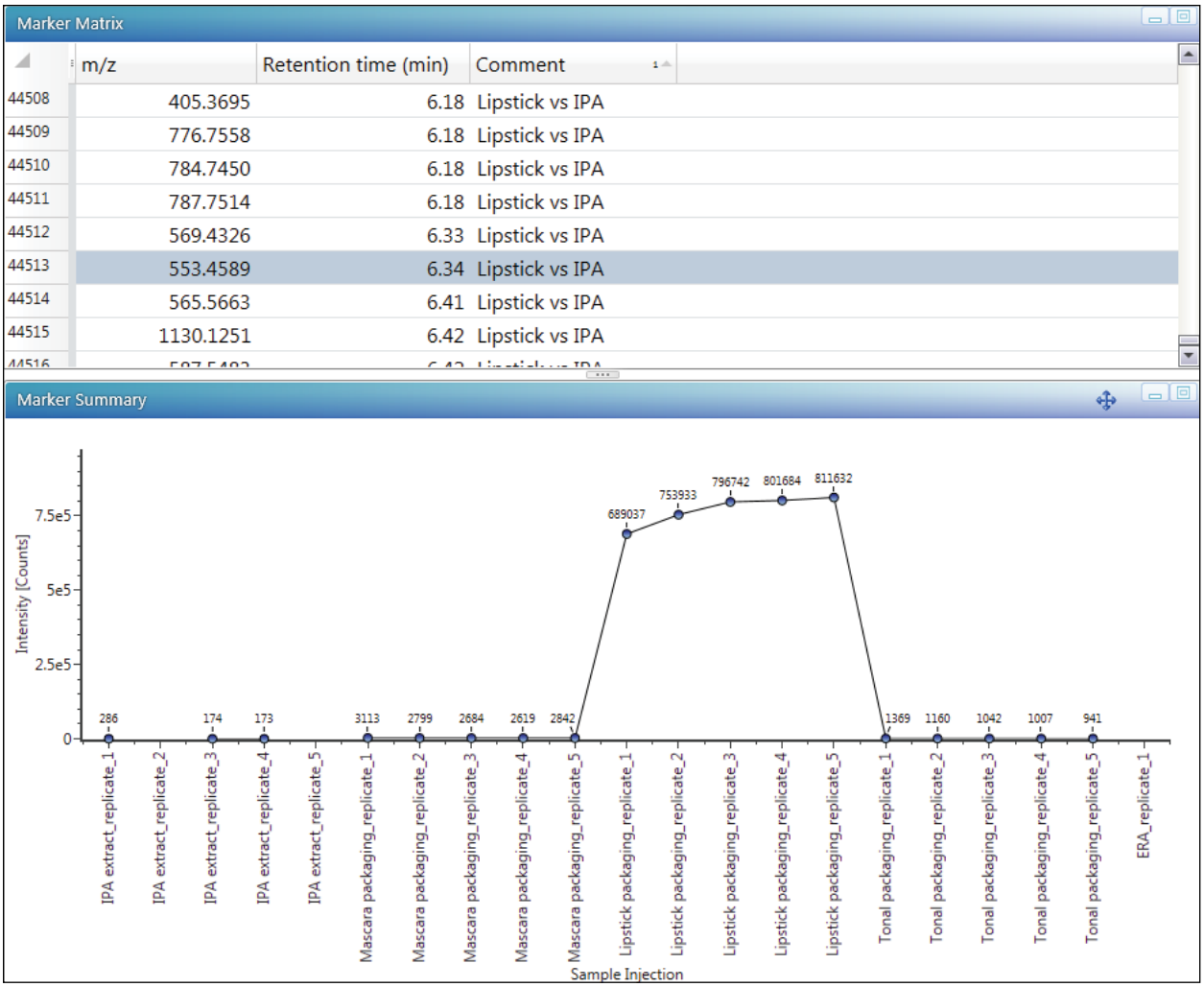

Figure 5. Marker Matrix with labeled markers and a trend plot for a marker 553.4589 at RT 6.34 min.

Discovery tool

Regardless of whether a marker or candidate of interest was obtained by binary compare or multivariate analysis, the next step in the workflow is structural elucidation. The Discovery tools within UNIFI's Elucidation toolset include automated elemental composition, database searching through ChemSpider or UNIFI's configurable Scientific Library, as well as fragment matching of high-collision energy data (Figure 6) of individual or batches of candidates. The best matches are displayed based upon the number of identified high energy fragments, citations from ChemSpider, and mass accuracy. The elemental composition algorithm uses accurate mass and isotope information to calculate the possible compositions for each marker. Using the Discovery tool settings, analysts can specify an acceptable level of isotope match (i-FIT™), elements to be included in the elemental composition search, which libraries to select from ChemSpider (all or specific ones), and minimum number of citations in ChemSpider, among other things.

The final results for the candidate mass *m/z* 360.3236 in the mascara packaging are displayed in a table that lists the elemental compositions within specified limits, possible structures with citations from the ChemSpider database, and how many fragments can be matched to the high collision energy data for each structure (Figure 7).

Many polymer additives form adducts during LC-MS (Na+ being the most common). The adduct ion can be more intense than the protonated species, or the protonated ion can be absent entirely. In this case, the initial evaluation of the mass using +H ion, did not provide a reasonable molecular formula (no i-FIT above 50% and no structure from ChemSpider). Therefore Na+ was selected as an adduct and the Discovery tool process was repeated. As shown in Figure 7, the molecular formula C22H43NO has a 100% i-FIT, meaning that the isotope ratio for the *m/z* is consistent with the proposed composition. ChemSpider returned a lot of possible structural hits for this formula. When sorted by the number of citations, it can be seen that the top choice also has one of the highest number of possible fragment matches in the high energy data. Additionally, common names are returned from the ChemSpider search that can help analysts determine the correct structure. Many polymer additives have common names such as Irganox's or Tinuvin's which are much easier to recognize than just a chemical name. The most cited chemical with the elemental composition C22H43NO has several common names indicating a polymer additive *e.g.* Armoslip E. Researching the identity of the chemical further, it turned out to be erucamide – a fatty acid derivative that is commonly used as a slip agent in packaging materials.

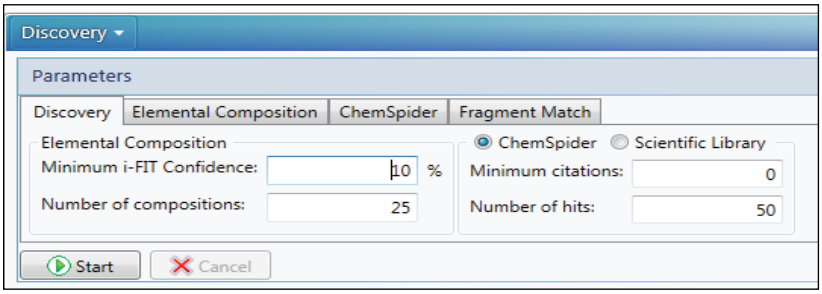

Figure 6. Interface for UNIFI's Discovery tool.

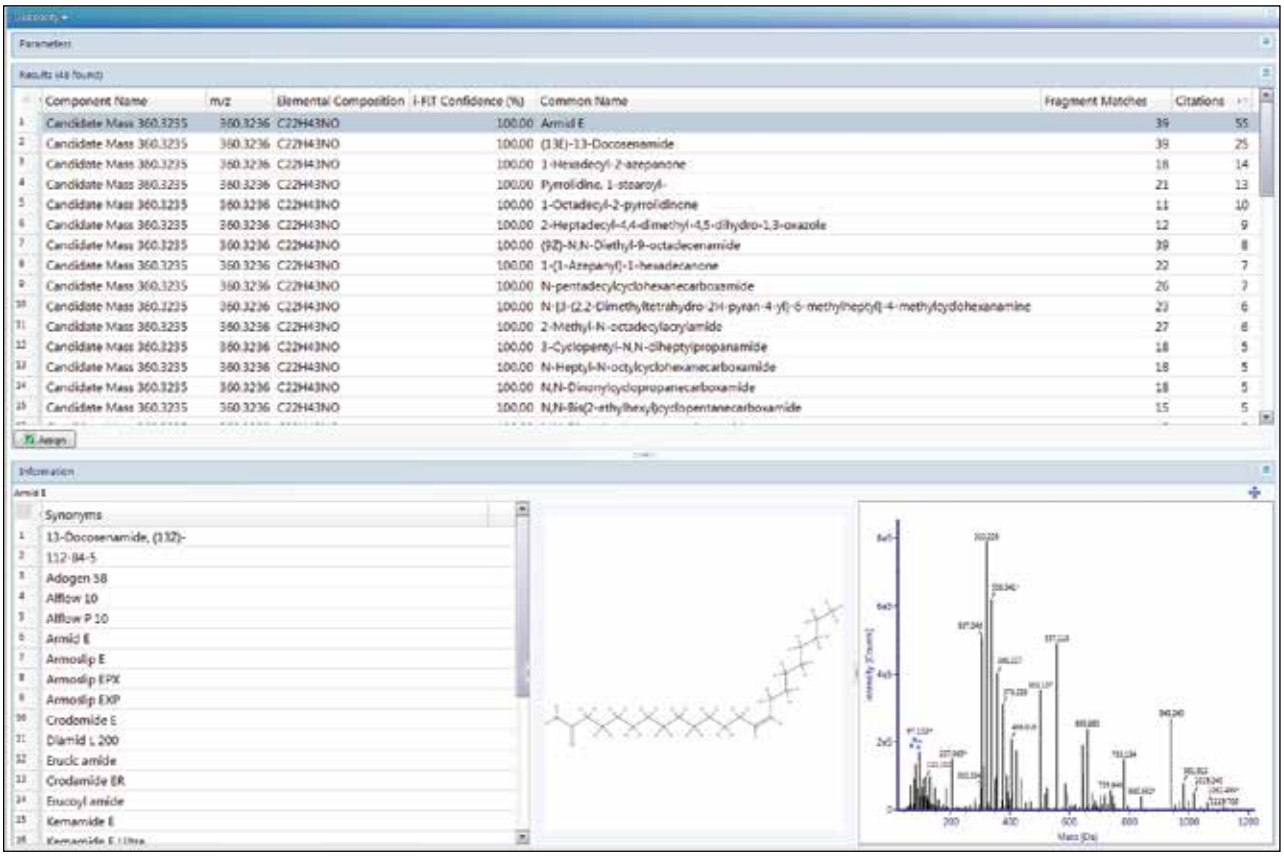

Figure 7. Results from UNIFI's Discovery tool for m/z 360.3236 at RT 4.18 in the mascara packaging.

CONCLUSIONS

Characterizing component spectra in non-optimized LC-MS analysis can be complex, therefore it is advantageous to use automated software tools to quickly evaluate possible structures for candidate masses. The described LC-MS and Informatics workflow, which employs high-resolution mass spectrometry, can be adopted for cosmetics, food, and pharmaceutical packaging extractable applications. Utilization of MS^E data containing accurate mass precursor and fragment ion information on a single software platform streamlines the identification and review process.

An Informatics-based structural elucidation discovery tool provides a rapid process to evaluate information for an unknown *m/z* by ranking the possible elemental compositions and subsequently searching databases for possible structures that are prioritized based on fragmentation matching. The UNIFI Software workflow makes it easy to rank markers of importance and facilitates component identification.

References

1. Council Directive 82/711/EEC of 18 October 1982 laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs, http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31982L0711
2. *Official Journal of the European Union.* 2011, Regulation 10/2011/EU.
3. D L Norwood, L M Nagao, C L M Stults. *PDA Journal of Pharmaceutical Science and Technology,* 67 (5): 413–429. 2013.
4. M P Balogh. Testing the Critical Interface: Leachables and Extractables. *LCGC*, June 1, 2011.
5. F Mofatt. Extractables and leachables in pharma-Serious Issue. http://www.solvias.com/sites/default/files/solvias_whitepaper_web.pdf
6. The European Parliament and the Council of the European Union. Regulations (EC) No 1223/2009 of the European Parliament and of the Council.

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Analysis of Primary Aromatic Amines in Cosmetics and Personal Care Products Using the ACQUITY UPLC H-Class System with the ACQUITY QDa Detector and Empower 3 Software

Jane Cooper
Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

ACQUITY® QDa® linked to the ACQUITY UPLC® H-Class System provides improved confidence in the identification and quantification of Primary Aromatic Amines (PAAs) in cosmetics and personal care products offering:

- The ultimate in chromatographic resolution and sensitivity.
- Increased sample throughput and a reduction of solvent usage due to reduced run times.
- Improved sensitivity, selectivity, and robustness, compared with existing methodologies.
- Cost-effective, reliable mass confirmation.

WATERS SOLUTIONS

ACQUITY UPLC H-Class System

ACQUITY QDa Detector

Empower® 3 Chromatography Data Software

KEY WORDS

Primary aromatic amines, PAAs, azo dyes, cosmetics, personal care products

INTRODUCTION

Primary aromatic amines (PAAs) have been broadly used in large amounts as a chemical feedstock within the chemical industry. Many PAAs have either a proven or suspected carcinogenic nature and are rated as highly toxic,[1,2,3] so there are a range of potential health risks, which have led to worldwide regulations. In the EU Cosmetic Regulations (EC) No 1223/2009,[4] many PAAs are prohibited for use in cosmetic products.

Despite the toxic and carcinogenic nature of PAAs, they are an important feedstock used in the production of many commodity products such as pharmaceuticals, pesticides, explosives, epoxy polymers, rubber, aromatic polyurethane products, and azo dyes. While not desirable in final products, the presence of PAAs may be due to incomplete reactions, impurities, by-products, or as degradation products. For example PAAs can be produced as by-products of azo dyes which are a diverse and extensively used group of organic dyes. Azo dyes are used in special paints, printing inks, varnishes and adhesives, and can be found in many products such as textiles, cosmetics, personal care products, plastics, and also in food contact material.

In order to ensure public safety and product efficacy, the cosmetics and personal care industry is highly legislated. Hence, manufacturers who use feedstock materials such as PAAs in the production of their products must monitor and quantify various regulated parameters, such as the presence or absence of PAAs.

Previous example methodologies for the analysis of PAAs include:

- GC/MS analysis following ion-pair extraction with bis-2-ethyl phosphate followed by derivatization with isobutyl chloroformate;[5,6]
- UPLC® analysis following a solid phase extraction (SPE) using cation-exchange cartridges;[7]
- reduction by liquid phase sorbent trapping followed by thermal desorption GC/MS analysis.[8]

However, many previously used methods for PAA analysis lack robustness, selectivity and sensitivity, and require lengthy, costly, and time-consuming pre-treatments (derivatization, SPE).

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC H-Class
Runtime:	10.00 min
Column:	ACQUITY BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Column temp.:	40 °C
Sample temp.:	10 °C
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Methanol + 0.1% formic acid
Flow rate:	0.4 mL/min
Injection volume:	10.0 µL

Mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.400	95	5	–
2	1.00	0.400	95	5	6
3	3.10	0.400	75	25	6
4	6.10	0.400	59	41	6
5	8.00	0.400	0	100	6
6	9.00	0.400	0	100	6
7	9.01	0.400	95	5	6
8	10.00	0.400	95	5	6

Table 1. ACQUITY UPLC H-Class mobile phase gradient.

MS conditions

Mass detector:	ACQUITY QDa
Ionization mode:	ESI +
Capillary voltage:	0.8 kV
Probe temp.:	450 °C
Acquisition:	Selected Ion Recording (SIR)
Cone voltage:	15 V

The list of PAAs, associated CAS number, *m/z*, and expected retention times, are detailed in Table 2.

An ideal solution for the cosmetic and personal care industry for the analysis of PAAs, would overcome the limitations of prior methodologies, while ensuring confidence and versatility in order to meet the regulatory requirement.

This application note describes an accurate, fast, and robust alternative method for the rapid analysis of PAAs in cosmetic and personal care products, using Waters® ACQUITY UPLC H-Class System coupled with the ACQUITY QDa Detector, and controlled by Empower 3 Software.

Instrument control, data acquisition, and result processing

Empower 3 Software was used to control the ACQUITY UPLC H-Class System and the ACQUITY QDa Detector, as well as for data acquisition and quantitation.

Sample preparation

Cosmetic and personal care product sample analysis (eyeshadow, blush, shampoo)

- 0.5 g (solid samples) or 0.5 mL (liquid samples), add 8 mL water and 2 mL methanol. Vortex mixture for 2 min (1600 rpm).
- Centerfuge approximately 1 mL extract for 5 min (10,000 rpm).
- Centrifuge extract diluted with methanol in LC vials ready for analysis (250 µL extract plus 750 µL methanol).

PAA number	Primary Aromatic Amines (PAAs)	CAS number	*m/z*	Retention time (min)
1	Aniline	62-53-3	94	0.47
2	o-Toluidine	95-53-4	108	0.96
3	1,3-Phenylenediamine	108-45-2	109	0.33
4	2,4-Dimethylaniline	95-68-1	122	2.55
5	2,6-Dimethylaniline	87-62-7	122	3.04
6	2,4-Toluenediamine	95-80-7	123	0.40
7	2,6-Toluenediamine	823-40-5	123	0.34
8	o-Anisidine	90-04-0	124	0.82
9	4-Chloroaniline	106-47-8	128	1.84
10	2-Methoxy-5-methylaniline	120-71-8	138	2.53
11	4-Methoxy-m-phenylenediamine	615-05-4	139	0.38
12	2-Naphtylamine	91-59-8	144	3.71
13	3-Amino-4-methylbenzamide	19406-86-1	151	0.71
14	3-Chloro-4-methoxyaniline	5345-54-0	158	1.45
15	5-Chloro-2-methoxyaniline	95-03-4	158	4.70
16	1,5-Diaminonaphtalene	2243-62-1	159	0.43
17	2-Methoxy-4-nitroaniline	97-52-9	169	4.62
18	4-Aminobiphenyl	92-67-1	170	5.62
19	2-Aminobiphenyl	90-41-5	170	6.83
20	Benzidine	92-87-5	185	0.42
21	4-Chloro-2,5-dimethoxyaniline	6358-64-1	188	4.76
22	4-Aminoazobenzol	60-09-3	198	8.14
23	4,4'-Methylenedianiline	101-77-9	199	0.67
24	3,3'-Dimethylbenzidine	119-93-7	213	2.37
25	4,4'-Thioaniline	139-65-1	217	3.98
26	o-Aminoazotoluene	97-56-3	226	8.62
27	4,4'-Diamino-3,3'-dimethylbiphenylmethane	838-88-0	227	3.32
28	3-Amino-p-anisanilide	120-35-4	243	5.10
29	o-Dianisidine	119-90-4	245	2.61
30	4,4'-Diamino-3,3'-dichlorobiphenylmethane	101-14-4	267	8.18

Table 2. PAAs, associated CAS number, m/z, and expected retention times.

RESULTS AND DISCUSSION

Optimum UPLC and SIR conditions were developed, with the elution of all compounds occuring within a 10 minute run. The speed of method development was markedly improved using the ACQUITY QDa Detector instead of UV detection.

Typically during method development, different conditions/parameters are considered such as choice of columns, mobile phases, and gradients. These choices could potentially result in changes to the elution order of the compounds being considered. The peak tracking when using UV detection only would require the analysis of the individual authentic standards in order to confirm the elution order (Rt). However, with mass detection, the movement of chromatographic peaks can easily be followed, and the presence of co-eluting peaks can also be easily identified.

An illustration of the identification of the co-eluting peaks is shown in Figure 1 which shows two PAAs (4,4'-Methylene-Dianiline and 2-Methoxy-5-Methylaniline) that have similar optimum wavelengths.

Mixed calibration standards, over the range of 0.001 µg/mL to 1.0 µg/mL were prepared and analyzed for all the PAAs considered (equivalent range of 0.08 to 80 mg/Kg in the extracted sample, using the developed method, greater with extract dilution). The SIR chromatograms for each PAA are shown in Figure 2.

The SIR mass detection conditions detailed in Table 2 were used after appropriate sample preparation to screen for PAAs in cosmetic and personal care samples.

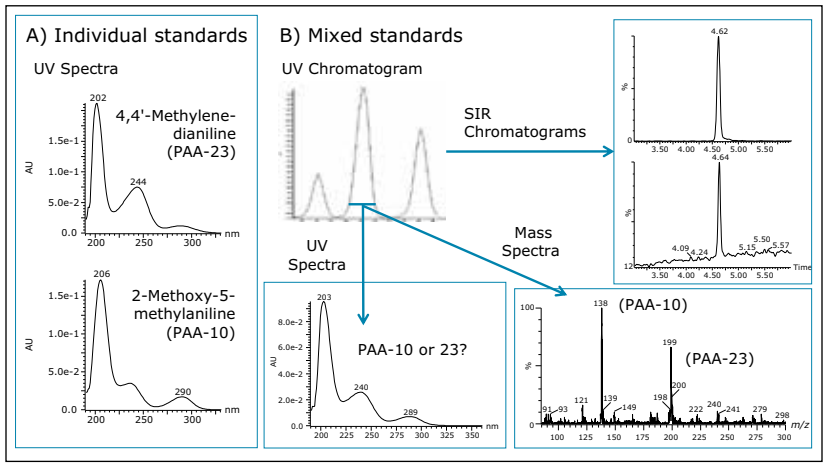

Figure 1. An illustration of the advantages of mass detection for the identification of co-eluting peaks during method development, considering two PAAs (4,4'-Methylene-dianiline and 2-Methoxy-5-methylaniline); a) UV spectra from individual standards, b) UV and mass spectra, and SIR chromatograms from mixed standards.

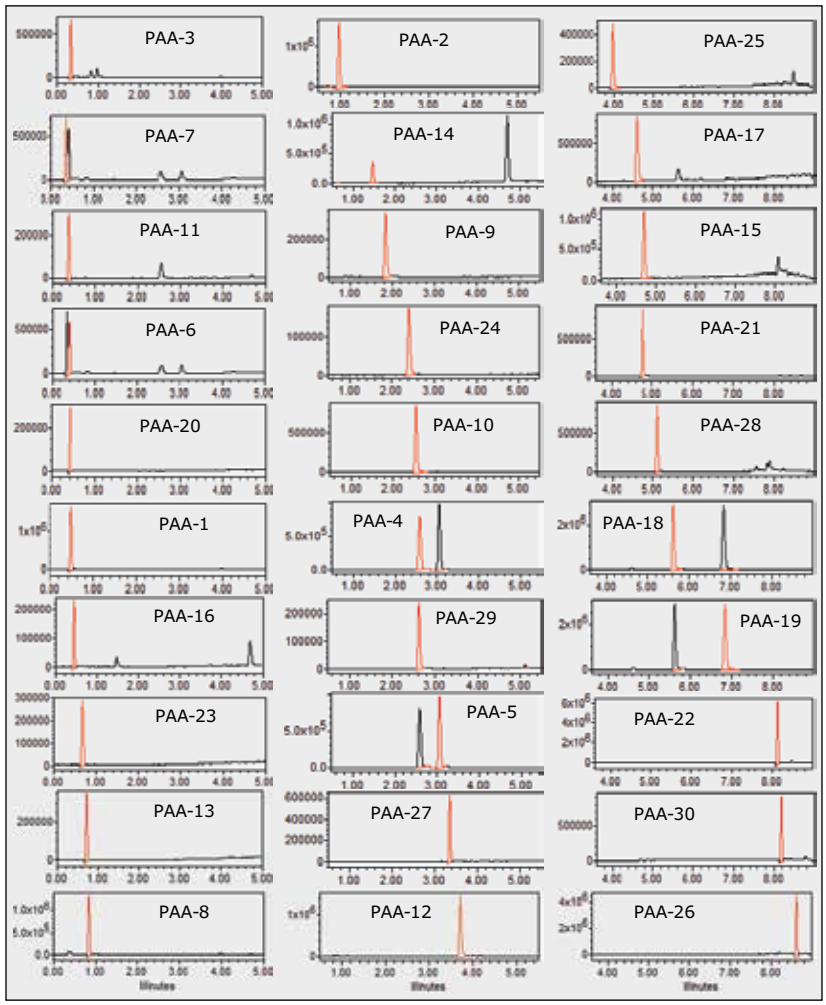

Figure 2. SIR chromatograms for 30 PAAs in a mixed 0.5 µg/mL calibration standard.

Cosmetic and personal care sample analysis

Samples were fortified at various levels with selected PAAs, then prepared for analysis as described in the Experimental section. The results obtained for shampoo, blush, and eyeshadow are detailed in Tables 3, 4, and 5, and a selection of SIR chromatograms achieved are shown in Figure 3.

Amine	Fortified mg/Kg	mg/Kg	Recovery (%)*
Aniline	0	0.012	N/A
	0.25	0.213	80.5%
	0.5	0.371	71.8%
	1.0	0.831	81.8%

*Table 3. Shampoo fortified at various levels with aniline. Results quantified against mixed calibration standards. *Blank corrected recovery data.*

Amine	Fortified mg/Kg	mg/Kg	Recovery (%)*
2,6-Dimethylaniline	0	0.018	N/A
	0.25	0.202	73.6
	0.5	0.417	84.0
	1.0	0.895	90.4
4-Chloroaniline	0	0.045	N/A
	0.25	0.222	70.8
	0.5	0.429	76.8
	1.0	0.785	74.0
2-Naphthylamine	0	ND	N/A
	0.25	0.254	101.6
	0.5	0.404	80.8
	1.0	0.865	86.5

*Table 4. Blush fortified with various levels of selected PAAs. Results quantified against mixed calibration standards. *Blank corrected recovery data.*

The recoveries obtained (ranging between 72% to 104%) demonstrated that minimal signal enhancement/ suppression was observed using UPLC chromatographic separation with ESI ionization for the analysis of PAAs in the cosmetic and personal care products considered.

Amine	Fortified mg/Kg	mg/Kg	Recovery (%)*
2,6-Dimethylaniline	0	ND	N/A
	0.25	0.207	82.8
	0.5	0.353	70.6
	1.0	0.775	77.5
4-Chloroaniline	0	0.095	N/A
	0.25	0.354	103.6
	0.5	0.455	72.0
	1.0	0.857	76.2
5-Chloro-2-methyoxyaniline	0	0.069	N/A
	0.25	0.268	79.6
	0.5	0.510	88.2
	1.0	0.893	82.4

*Table 5. Eyeshadow fortified with various levels of selected Primary Aromatic Amines. Results quantified against mixed calibration standards. *Blank corrected recovery data.*

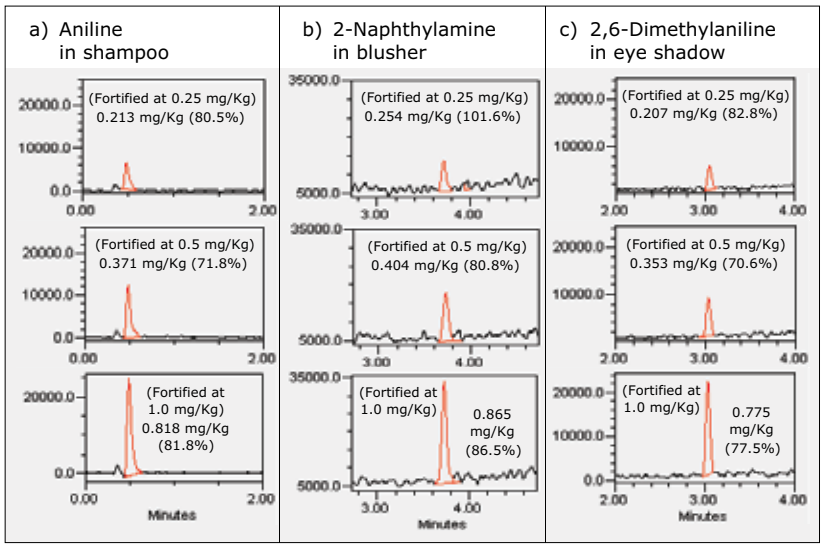

Figure 3. SIR chromatograms for selected PAAs in matrix: a) shampoo b) blush, and c) eyeshadow.

CONCLUSIONS

- A fast, robust, and sensitive method has been developed for the analysis of PAAs in cosmetic and personal care product samples.
- The ACQUITY QDa Detector provides more cost-effective and reliable mass confirmation, demonstrating improved experimental confidence over UV detection, during both method development and routine analysis.
- Combining the ACQUITY UPLC H-Class System with the ACQUITY QDa Detector offers accurate and reproducible quantification.
- Empower 3 Chromatography Data Software provides assurance in data management, data processing, and reporting.
- Business benefits compared to previous methodology include:
  - Increased sample throughput
  - Reduction of solvent usage due to no time-consuming derivatization or pre-concentration steps.
  - Reduced run times.
- The ACQUITY H-Class System, a quarternary system based on UPLC Technology, offers the best in chromatographic resolution and sensitivity.

References

1. Benigni R, Passerini L. Carcinogenicity of the aromatic amines: from structure-activity relationships to mechanisms of action and risk assessment. Mutation Research. 511: 191–206; 2002.
2. Anirban M.P, Cote R.J. Molecular Pathogenesis and Diagnosis of Bladder Cancer. Annual Review of Pathology. 4: 501–506; 2009.
3. Ward E, Carpenter A, Markowitz S, et al. Excess Cancers in Workers Exposed to Ortho-Toluidine and Aniline. National Cancer Institute. 83(7): 501–506; 1991.
4. The European Parliament and the Council of the European Union. Regulations (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. *Official Journal of the European Union*. L 342/59: 59–209, 22nd Dec 2009. [cited 2015 January 15]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF
5. Akyuz M, Ata S. Determination of aromatic amines in hair dye and henna samples by ion-pair extraction and gas chromatography-mass spectrometry. *J Pharm Biomed Anal.* 47, 68; 2008.
6. Ekladius L, King H K. A colorimetric method for the determination of aliphatic amines in the presence of ammonia. *J Chrom A.* 1129(1). Epub 2006 Jul 14.
7. Aznar M, Canallas E, Nerin J. Quantitative determination of 22 primary aromatic amines by cation-exchange solid-phase extraction and liquid chromatography-mass spectrometry. *J Chrom A*. 2009; 1216: 5176–5181; 2009.
8. Zhang Q, Wang C, et al. Determination of aromatic amines from azo dyes reduction by liquid-phase sorbent trapping and thermal desorption-gas chromatography-mass spectrometry. *J Sep Sci*. 32: 2434–2441; 2009.

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High Throughput Analysis of Phthalates and Parabens in Cosmetics and Personal Care Products Using UPLC with Mass Detection and Empower 3 Software

Jane Cooper
Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

The ACQUITY® QDa® Detector linked to the ACQUITY UPLC® H-Class System provides improved confidence in the identification and quantification of phthalates and parabens in cosmetics and personal care products offering:

- Increased sample throughput and a reduction of solvent usage due to reduced run times.
- Improved sensitivity, selectivity, and robustness, compared with existing methodologies.
- Cost effective, reliable mass confirmation.

WATERS SOLUTIONS

ACQUITY UPLC H-Class System

ACQUITY UPLC BEH Column

ACQUITY QDa Detector

Empower® 3 CDS Software

KEY WORDS

Phthalates, parabens, triclocarban, consumer products, cosmetics, personal care products

INTRODUCTION

Phthalates are esters of phthalic acid that have extensively been used as plasticizers to increase flexibility, transparency, durability, and longevity in a wide variety of consumer and household products, such as children's toys, electronics, clothes, flooring, wallpaper, and paints. Phthalates are also used, as plasticizers, solubilizers, or denaturants in cosmetics and personal care products, such as perfumes, nail polishes, and hair sprays.

Parabens are esters of parahydroxybenzoic acid, which due to their low volatility, high stability, antibacterial and antifungal properties, have been used as preservatives in cosmetics, personal care, pharmaceutical, food, and industrial products.

Triclocarban is an antibacterial and antifungal agent that is used in many cosmetic and personal care products, including soap, toothpaste, deodorant, shampoo and shaving cream. Triclocarban is also used in several consumer products including kitchen cutting boards, shoes, towels, and clothing, as well as in medical disinfectants and medical products. But there are several health concerns related to the use of triclocarban, including potential hormone and endocrine disruption, and also its potential to contribute to the development of antibiotic resistance.

Many phthalates are classified as hazardous because of their effects on the reproductive system and their association with an increased risk of cancer. Parabens are associated with allergenic contact dermatitis and rosecea. Studies[1,2] have also suggested parabens may be carcinogenic and possess estrogenic disrupting activities. Due to these properties phthalates, parabens, and triclocarban are either banned or restricted, as regulated by the Cosmetic Directive 1223/2009.[3]

In order to accommodate consumer demands for higher standards, many manufacturers are developing, and labeling cosmetic and personal care products 'free from' phthalates and parabens.

Previous example methodologies for the analysis of phthalates include GC-MS,[4] and HPLC-UV[4]; GC-FID,[5] HPLC-UV,[4,6] HPLC-MS,[7] GC-MS,[4] and capillary electrophoresis[6] for the analysis of parabens; and HPLC-MS[8] for the analysis of triclocarban.

Accessible and intuitive as an optical detector, the ACQUITY QDa Detector has been designed for chromatographers with ease of use in mind. Mass detection can be used to achieve reliable analytical methods to unequivocally identify and quantify compounds such as phthalates, parabens, and triclocarban, during both method development stages, and during routine regulatory analysis.

This application note considers the method development, sample extraction, and mass spectral analysis of parabens, phthalates, and triclocarban using Waters® ACQUITY UPLC H-Class System, coupled to the ACQUITY QDa Detector.

EXPERIMENTAL

Sample preparation

Cosmetic and personal care sample analysis

- Add 2.5 mL water and 2.5 mL methanol to 0.2 g sample.
- Vortex mixture for 2 minutes (1600 rpm).
- Further extract mixture in an ultrasonic bath for 30 minutes.
- Centrifuge approximately 1 mL of extract for 5 min (10,000 rpm).
- Transfer centrifuge extract to LC vials for analysis.

LC conditions

LC system:	ACQUITY UPLC H-Class
Runtime:	5.00 min
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Column temp.:	40 °C
Sample temp.:	10 °C
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Methanol + 0.1% formic acid
Flow rate:	0.6 mL/min
Injection volume:	5.0 µL

Mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.60	30	70	–
2	1.00	0.60	30	70	6
3	1.50	0.60	10	90	6
4	4.00	0.60	10	90	6
5	4.01	0.60	30	70	6
6	5.00	0.60	30	70	6

Table 1. ACQUITY UPLC H-Class mobile phase gradient.

MS conditions

MS system:	ACQUITY QDa
Ionization mode:	ESI + and -
Capillary voltage:	0.8 kV
Probe temp.:	450 °C
Acquisition:	Selected Ion Recording (SIR)
Cone voltage:	15 V

The list of compounds considered, including phthalates, parabens, and triclocarban, along with their expected retention times are detailed in Table 2. The empirical formulas and structures are detailed in Tables 3 and 4.

	ESI ionization mode (-/+)	SIR (*m/z*)	Retention time (minutes)
Diethyl phthalate	+	223.1	0.37
Dipropyl phthalate	+	251.1	0.58
Dibutyl phthalate	+	279.2	1.12
Benzylbutyl phthalate	+	313.4	1.07
Bis(2-ethylhexyl) phthalate	+	391.3	2.92
Diisobutyl phthalate	+	279.2	1.04
Di-n-pentyl phthalate	+	307.2	2.10
Di-n-hexyl phthalate	+	335.2	2.44
Dicyclohexyl phthalate	+	331.2	2.09
Di-(2-methoxyethyl)-phthalate	+	283.1	0.28
Di-n-octyl phthalate	+	391.3	3.10
Methylparaben	-	151.1	0.27
Ethylparaben	-	165.0	0.30
Propylparaben	-	179.0	0.35
Butylparaben	-	193.1	0.44
4-Hydroxybenzoic acid	-	137.0	0.24
Benzylparaben	-	227.1	0.43
Triclocarban	-	315.0	1.07

Table 2. Phthalates, parabens, and triclocarban; ionization mode, SIR m/z, and expected retention times.

Instrument control, data acquisition, and result processing

Empower 3 Software was used to control the ACQUITY UPLC H-Class System and the AQCUITY QDa Detector, as well as for data acquisition and quantitation.

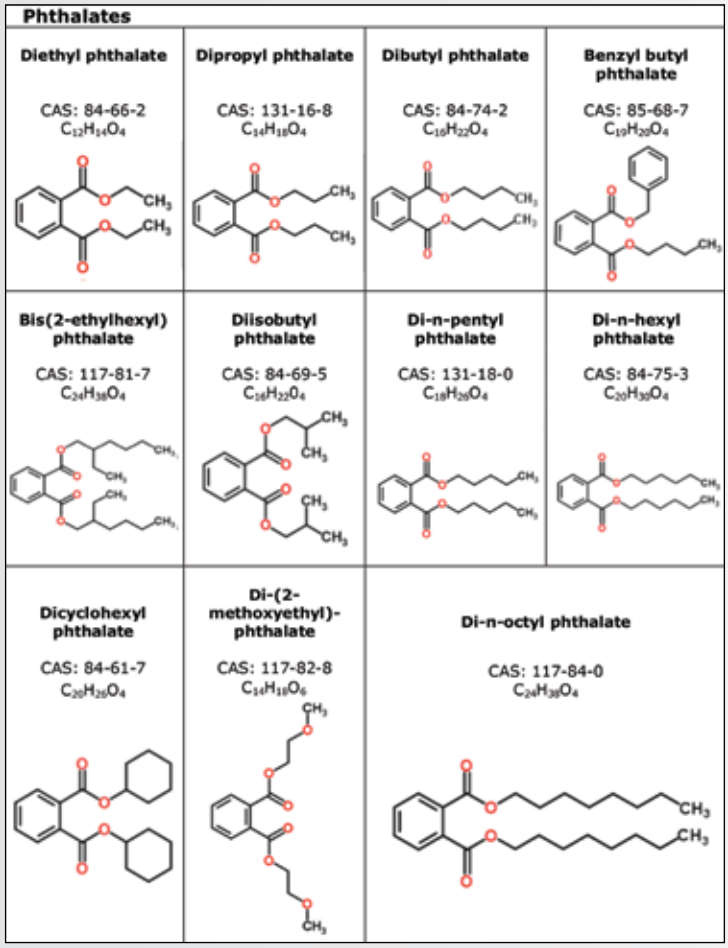

Table 3. Phthalates, associated CAS numbers, empirical formulas, and structures.

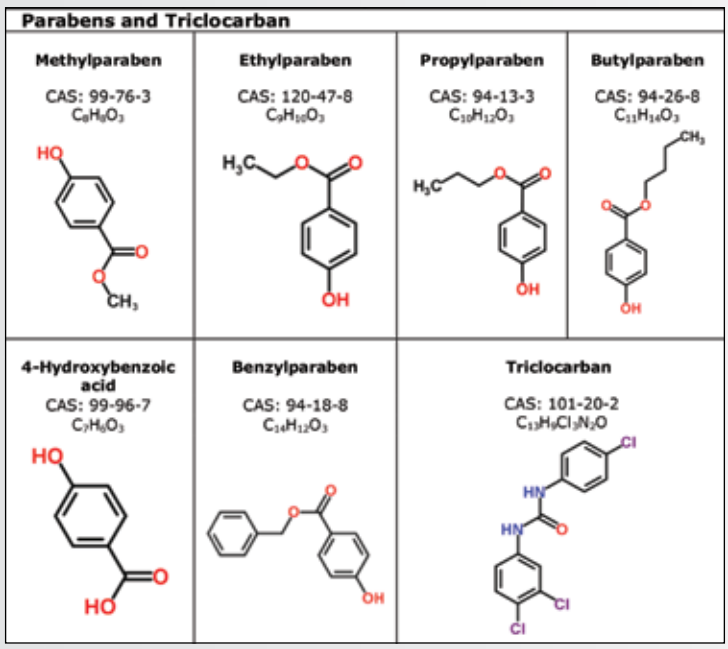

Table 4. Parabens and triclocarban, associated CAS numbers, empirical formulas, and structures.

RESULTS AND DISCUSSION

A fast, selective, and sensitive LC-MS method for the detection of a selection of phthalates, parabens, and triclocarban in cosmetic and personal care products has been developed.

The ACQUITY QDa Detector's SIR parameters were optimized, considering both negative and positive electrospray ionization modes, in order to ensure full coverage of all the compounds being analyzed (as detailed in Table 2.)

Method development was carried out using reversed phase UPLC,® where different gradient conditions, columns, and mobile phases were considered. The objective was to separate the isomeric phthalate compounds considered: di-n-octyl phthalate (DiNP), and diisobutyl phthalate (DiBP); bis(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP) – while maintaining sample throughput. This was achieved by optimizing the mobile phases and the gradient eluting conditions used. The final LC conditions used are detailed in the methods section.

The method was established over the calibration ranges of 0.01 µg/mL to 10 µg/mL for phthalates and triclocarban, and 0.05 µg/mL to 25 µg/mL for parabens, equivalent to 0.25 to 250 mg/Kg, and 1.25 to 625 mg/Kg in the extracted samples respectively. Good linearity was achieved for all the compounds considered (R^2 >0.99). SIR chromatograms for phthalates, parabens, and triclocarban in a mixed 1.0 µg/mL calibration standard are shown in Figure 1.

The developed five-minute UPLC method, is more than seven times faster than existing HPLC and GC methods, with an excess of 90% less solvent usage than existing HPLC methods.

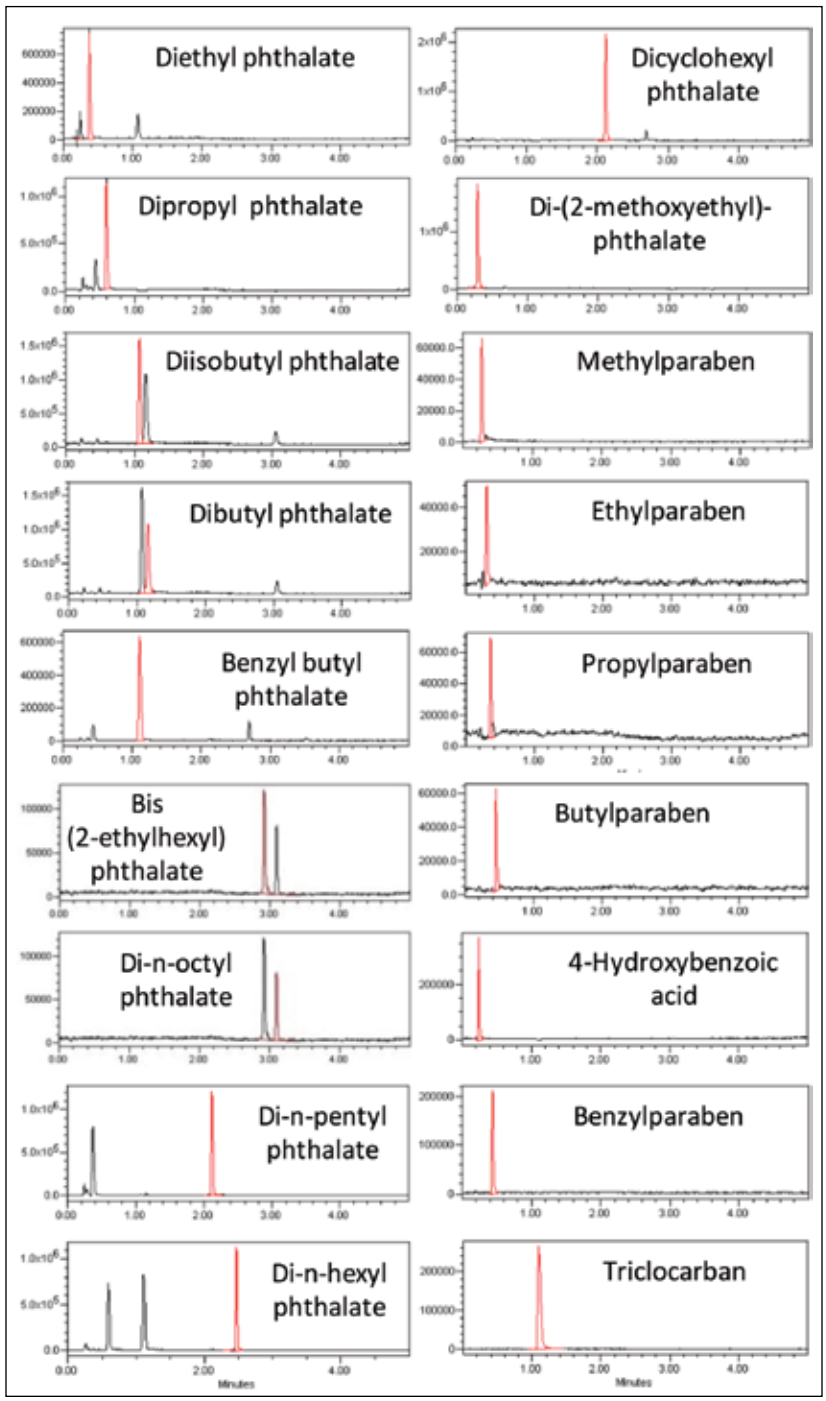

Figure 1. SIR chromatograms for phthalates, parabens, and triclocarban in a mixed 1.0 µg/mL calibration standard.

The SIR mass detection conditions detailed in Table 2 were used after appropriate sample preparation to screen for phthalates, parabens, and triclocarban in cosmetic and personal care samples.

Cosmetic and personal care sample analysis

Samples were fortified at various levels with selected phthalates and parabens, then prepared for analysis as detailed in the experimental section. Example SIR chromatograms achieved are shown in Figure 2.

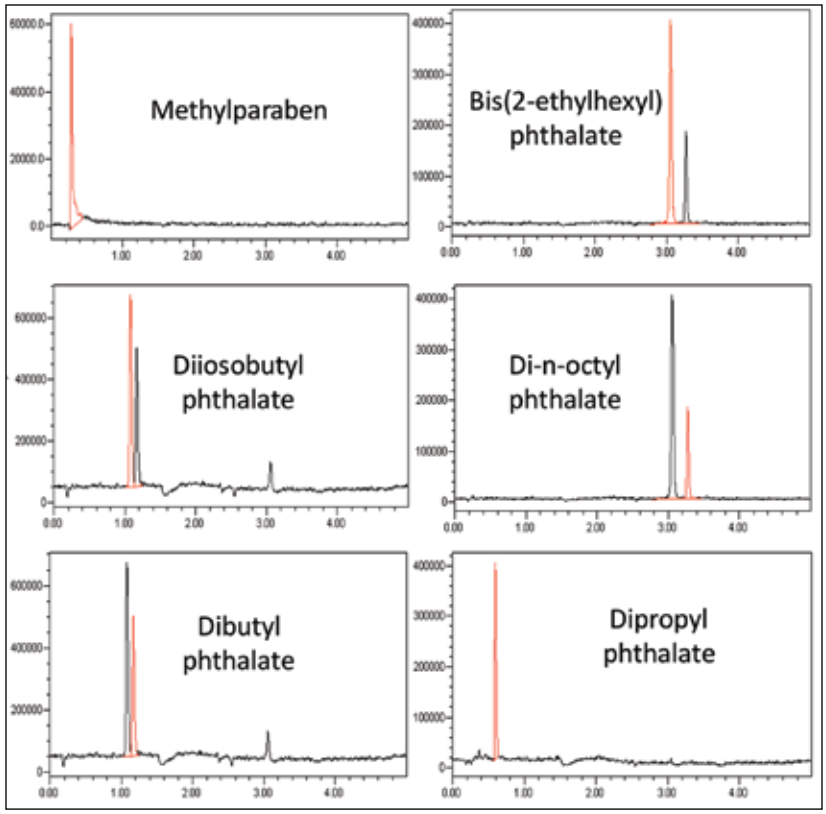

Figure 2. SIR chromatograms for selected phthalates and parabens in hair conditioner.

CONCLUSIONS

- A fast, robust, and sensitive method was developed for the combined analysis of phthalates, parabens, and triclocarban in cosmetic and personal care samples.
- The ACQUITY QDa Detector provides cost effective reliable mass confirmation, during both method development and routine analysis.
- Combining the ACQUITY UPLC H-Class System with the ACQUITY QDa Detector offers accurate and reproducible quantification.
- Empower Chromatography Data Software provides confidence in data management, data processing, and reporting.
- The developed 5-minute UPLC method is more than 7 times faster than existing HPLC and GC methods, with an excess of 90% less solvent usage than existing HPLC methods.
- The ACQUITY H-Class System, a quarternary system based on UPLC Technology, offers the best in chromatographic resolution, and sensitivity.

References

1. Darbe P D. Environmental oestrogens, cosmetics and breast cancer. *Best Practice & Research Clinical Endocrinology & Metabolism*. 20(1): 121–143, 2006.
2. Golden R, Gandy J, Vollmer G. A review of the Endocrine activity of parabens and implications for potential risks to human health. *Critical reviews in toxicology.* 35(5): 435–458, 2005.
3. The European Parliament and the Council of the European Union. Regulations (EC) No 1223/2009 of The European Parliament and of the Council of 30 November 2009 on Cosmetic Products. Official Journal of the European Union. L 342: 59–209, 30th November 2009. [cited 2015 August 25]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF
4. Shen H Y, Jiant H L, Mao H L, et al. Simultaneous determination of seven phthalates and four parabens in cosmetic products using HPLC-DAD and GC-MS methods. *J Sep Sci.* 30: 48–54, 2007.
5. Farajzadeh M A, Djozan Dj, et al. Use of a capillary tube for collecting an extraction solvent lighter than water after dispersive liquid-liquid microextraction and its application in the determination of parabens in different samples by gas chromatography-flame ionization detection. *Talanta*. 81: 1360, 2010.
6. Labat L, Kummer E, Dallet P, Dubost JP. Comparison of high-performance liquid chromatography and capillary zone electrophoresis for the determination of parabens in a cosmetic product. *J Pharm Biomed Anal.* 23:763, 2000.
7. González-Mariño I, Quintana J B, Rodríguez, Cela R. Evaluation of the occurrence and biodegradation of parabens and halogenated by-products in wastewater by accurate-mass liquid chromatography-quadrupole-time-of-flight-mass spectrometry (LC-QTOF-MS). *Water Research.* 20: 6770–6780, 2011.
8. Wang Y, Li P, Liu Y, et al. Determination of Triclocarban, Triclosan and Methyl-Triclosan in Environmental Water by Silicon Dioxide/Polystyrene Composite Microspheres Solid-Phase Extraction Combined with HPLC-ESI-MS. *Journal of Geoscience and Environment Protection.* 1(2): 13–17. 2013.

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Analysis of Disperse Dyes Using the ACQUITY Arc System with PDA and Mass Detection, and Empower Software

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[2]Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- Enhanced confidence in the profiling of impurities using PDA and mass detection.
- Ease of use with single point control via Empower® 3 Software.
- Dual-flow paths to emulate HPLC and UHPLC separations.

WATERS SOLUTIONS

ACQUITY® Arc™ System

2998 Photodiode Array (PDA) Detector

ACQUITY QDa® Detector

XBridge® C_{18} Column

Empower 3 CDS Software

KEY WORDS

Disperse dyes, consumer products, textile, impurity identification, mass detection

INTRODUCTION

Disperse dyes are low molecular weight synthetic dyes. The structure of the dyes can often contain azo or anthraquinone functional groups.[1] The primary application of disperse dyes is in consumer products such as textiles, paper, toys etc. Several of the dyes have been found to induce an allergic response as a result of prolonged exposure to the skin.[2] The presence of azo groups in the structure of some dyes provides the possibility for them to be converted to potential or known carcinogenic aromatic amines.[2]

The existence of these dyes in consumer products has led to increased awareness of the potential harmful effects to consumer health. Legislation controlling the use of several of these dyes was introduced in Germany in 1996. This led to the development of the DIN 54231 standard procedure which describes a method for the analysis of disperse dyes that employs high performance liquid chromatography (HPLC) or thin layer chromatography (TLC) with either ultraviolet (UV), mass spectrometry (MS), or densitometry detection for the analysis of the dyes.[3-5]

In this application note, we present the analysis of nine disperse dyes (Figure 1) using the standard DIN 54231 procedure with a combination of UV and mass detection, and a dual-flow path liquid chromatography system capable of emulating HPLC or UHPLC separations.[6] The inclusion of the mass detector allowed increased information to be derived from the analysis including confirmation of impurity peaks in specific dye samples. The detection limit when measured using the disperse blue 1 dye standard is specified as 0.7 mg/L in the DIN 54231 method. Using Waters® ACQUITY Arc System and the ACQUITY QDa Detector, the detection limit achieved significantly surpassed the specified detection limit for all compounds evaluated.

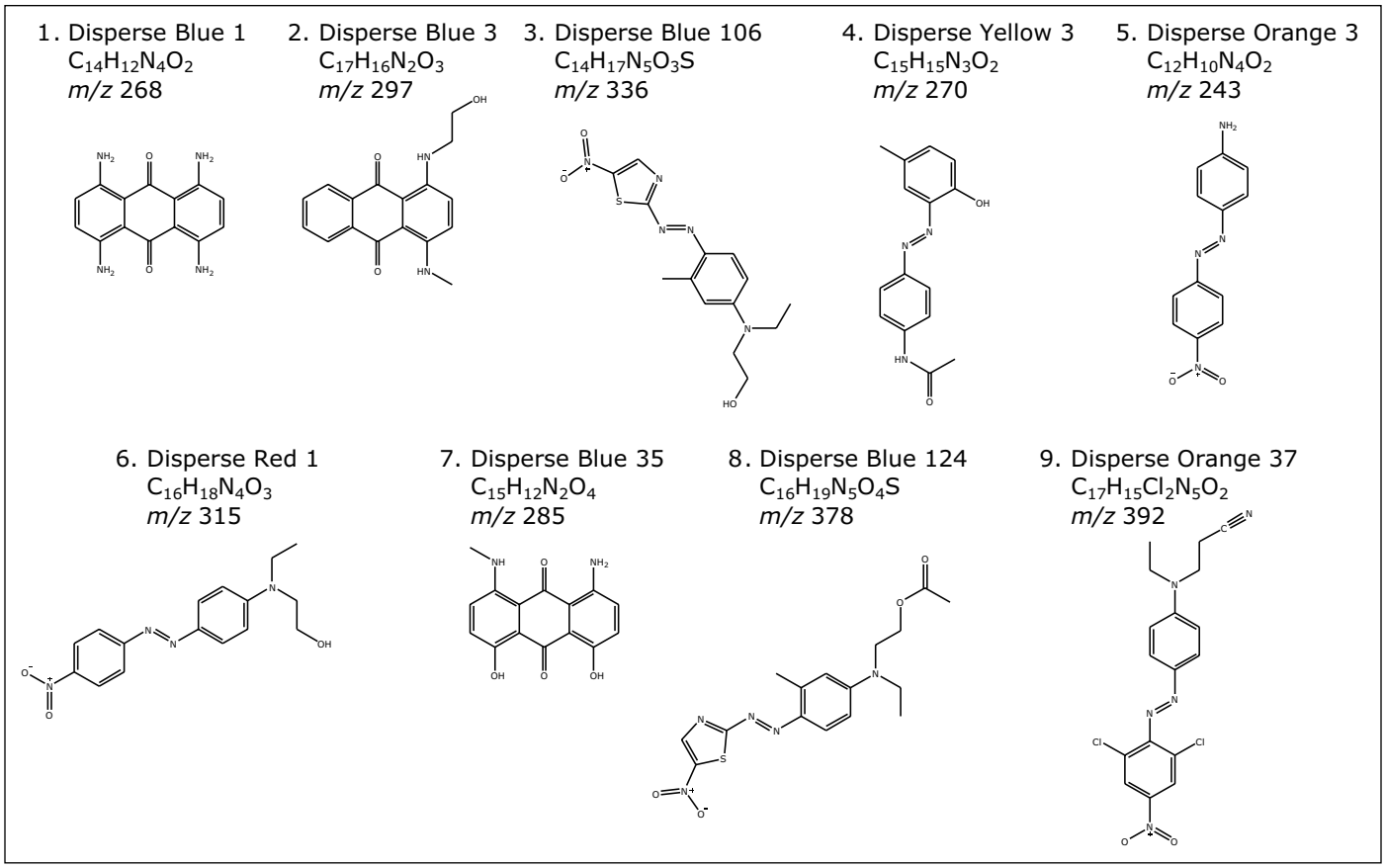

Figure 1. Empirical formulas, structures, and m/z for the disperse dyes used in this study.

EXPERIMENTAL

Instrumentation and software

All separations were performed on the ACQUITY Arc System equipped with a 2998 Photodiode Array (PDA) Detector and positive ion electrospray mass spectrometry (MS) using the ACQUITY QDa Detector. Empower 3 Software was used for data acquisition and processing.

Sample preparation

The dye standards were dissolved in methanol and sequentially diluted in preparation for sample analysis.

LC conditions

HPLC method (DIN 54231)

LC system:	ACQUITY Arc
Separation mode:	Gradient
Column:	XBridge C_{18}, 2.1 x 150 mm, 5 µm
Solvent A:	Ammonium acetate 10 mmol pH 3.6
Solvent B:	Acetonitrile
Flow rate:	0.30 mL/min
PDA detection:	210 to 800 nm
Column temp.:	30 °C
Injection volume:	5 µL
Analysis time:	30 min
Gradient conditions:	0 min 40% B, 7 min 60% B, 17 min 98% B, 24 min 98% B, return to initial conditions.

MS conditions

MS system	ACQUITY QDa
Ionization mode:	ESI +
Capillary voltage:	1.2 kV
Cone voltage:	10 V
Desolvation temp.:	600 °C
Source temp.:	150 °C
MS scan range:	100 to 600 *m/z* and Selected Ion Recording (SIR)
Sampling rate:	5 Hz

Figure 2 shows a PDA chromatogram at 240 nm resulting from the separation of a mixture of nine disperse dye standards (lower trace), and the superimposed SIR channels (top trace) obtained using a 2.1 x 150 mm, 5-µm XBridge C_{18} Column, (Part no. 186003110).

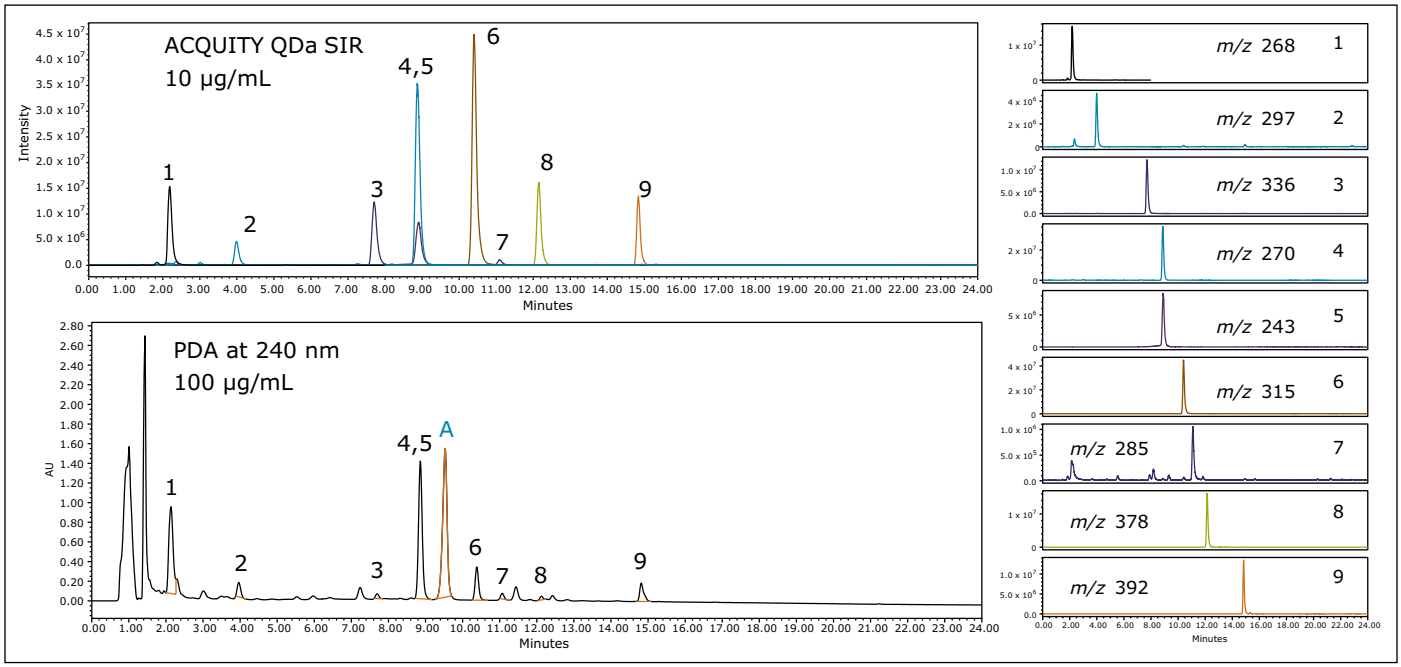

Figure 2. ACQUITY Arc chromatogram from the separation of nine disperse dye standards (100 µg/mL, 5 µL injection) at 240 nm using the DIN 54231 standard method and an XBridge C_{18}, 2.1 x 150 mm, 5.0-µm Column (lower). The superimposed (top) and the individual stacked (right) SIR channel chromatograms (10 µg/mL, 5 µL injection) are also shown.

Note that there is a coelution of the chromatographic peaks resulting from disperse yellow 3 (peak 4), and disperse orange 3 (peak 5) which makes accurate detection by UV alone challenging. Chromatographic separation of the components would be required for accurate detection if UV was to be used which would extend the method development time. The components have different *m/z* ratios, which enabled independent detection using the ACQUITY QDa despite the coelution, as can be seen from the stacked individual SIR chromatograms shown in Figure 2. Detection sensitivity was significantly improved using the mass detector.

Impurity analysis

A prominent peak (peak A) was detected in the PDA data at a retention time (t_R) of 9.5 minutes. This signal was absent from the SIR channels as the specific *m/z* for this unknown component was not monitored in the experimental method. An MS full scan experiment was performed simultaneously with the PDA detector making it possible to determine the mass spectra as well as the UV spectra for all components in the mixture (Figure 3).

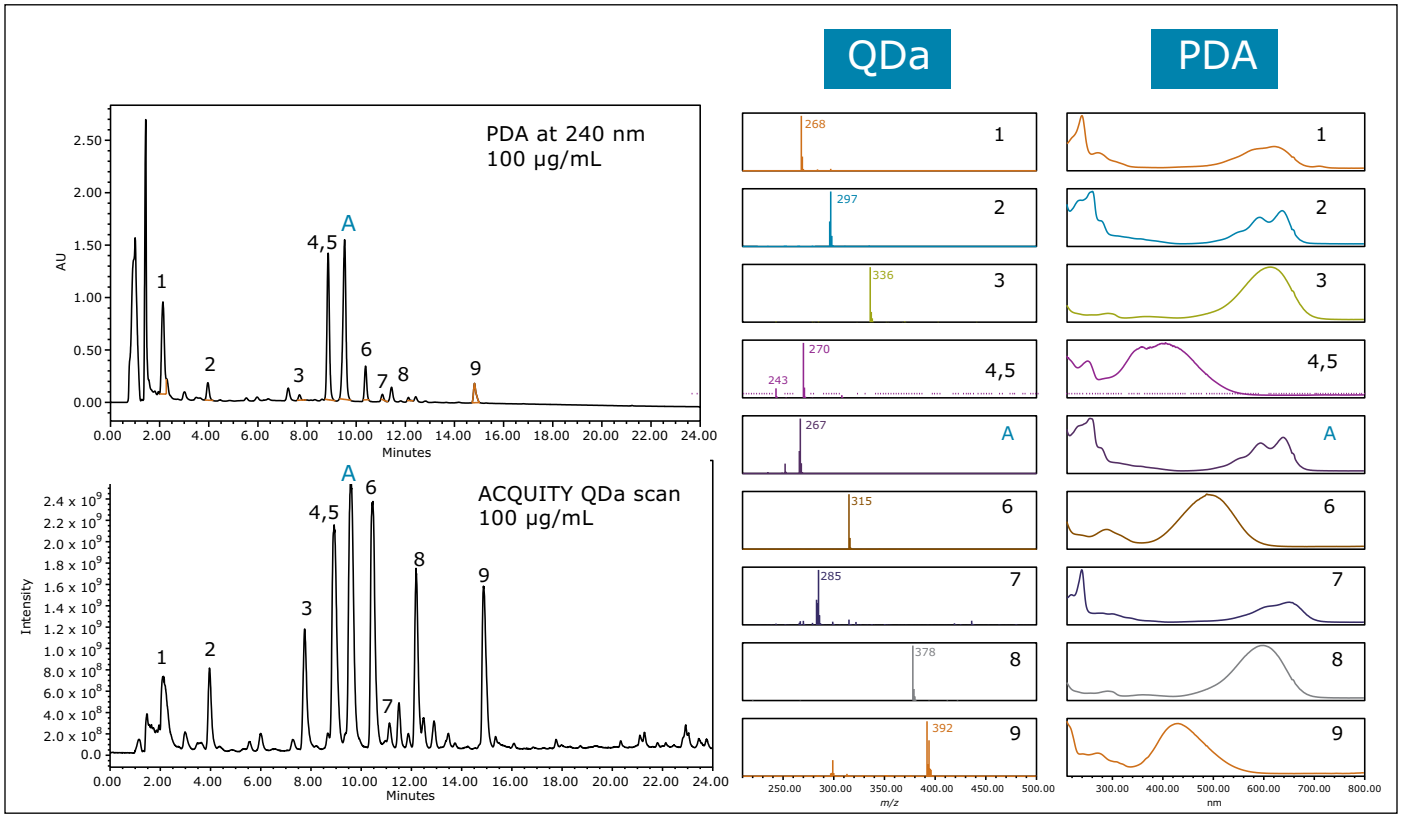

Figure 3. ACQUITY Arc chromatograms from the separation of nine disperse dye standards at 240 nm (top) (100 µg/mL, 5 µL injection) and QDa MS scan (100–600 m/z) (bottom) using the DIN 54231 standard method and an XBridge C_{18}, 2.1 x 150 mm, 5.0-µm Column. The MS and UV spectra are also shown.

The MS spectra for the unknown component A showed a large spectral peak with *m/z* 267. In addition the UV spectra of peak 2 which corresponds to disperse blue 3 and that of unknown peak A had similar features indicating that they may share common structural characteristics. A standard solution containing only disperse blue 3 which had a dye content of 20% was analyzed (Figure 4). The Mass Analysis window from Empower Software allowed rapid confirmation of the identity of disperse blue 3 (*m/z* 297) by displaying both the UV and mass spectra simultaneously. The mass spectrum for unknown peak A indicates that the base peak for this component is *m/z* 267 which matched the previous analysis of the mixture. In addition the t_R and the UV spectra were the same in both analyses. A second unknown component with a t_R of 11.4 minutes, labeled B, with an *m/z* 254 was also detected in the analysis of the disperse blue 3 dye standard. The ACQUITY QDa and PDA data provided complementary information which allowed us to conclude that the impurity A previously detected in the mixture of dyes originated from the disperse blue 3 standard.

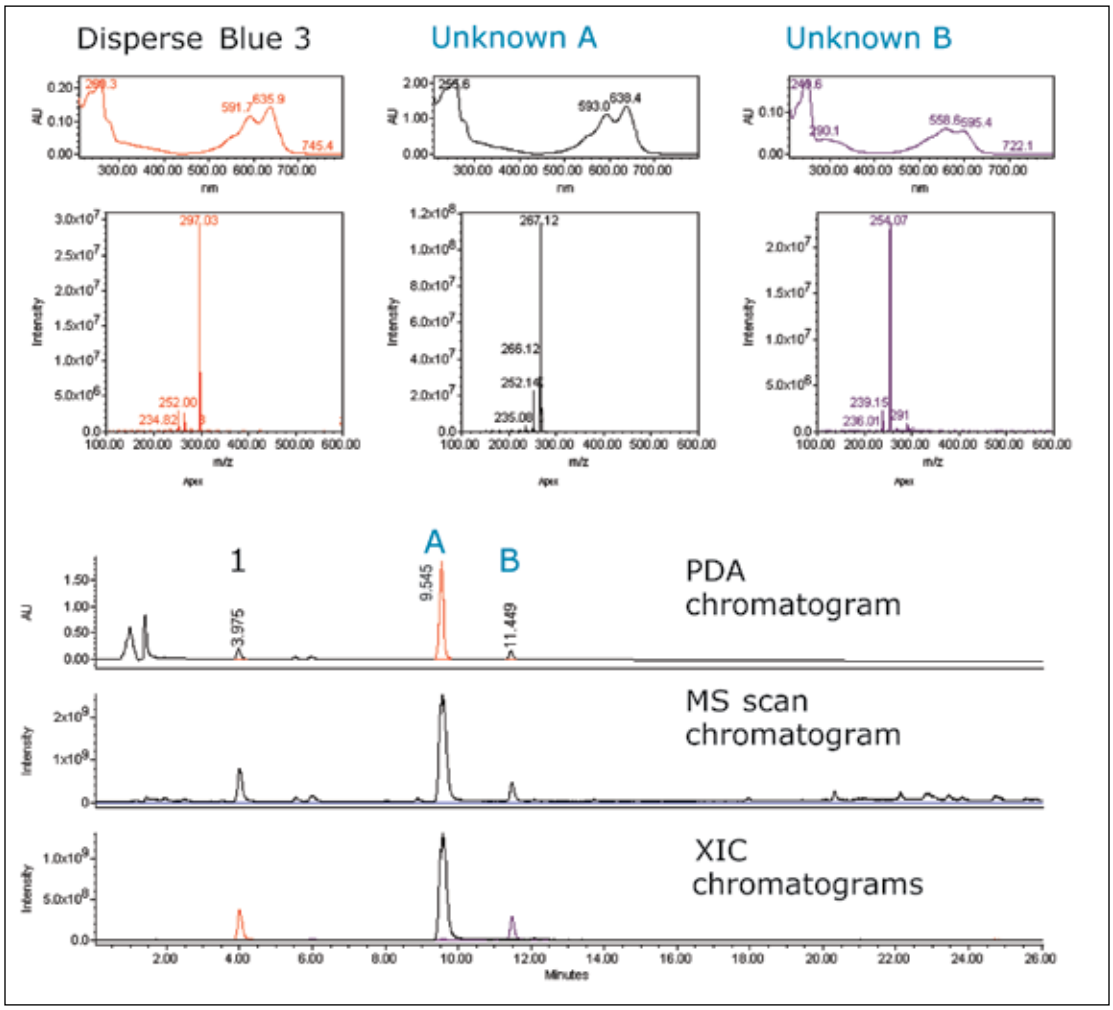

Figure 4. Empower Software's Mass Analysis window showing UV and MS spectra (top). ACQUITY Arc, PDA, MS scan (100–600 m/z) and superimposed XIC chromatograms of a single standard of disperse blue 3 using the DIN 54231 standard method.

CONCLUSIONS

The addition of mass detection as a complementary analytical detection technique enhances confidence in compound detection and identification. Co-eluting components with different *m/z* ratios can be reliably analyzed using mass detection. The detection limits required for the DIN method can be surpassed for all compounds using the described analytical methodology. The presence of both PDA and mass detection helped confirm that an impurity detected during method development originated in the disperse blue 3 standard. Thus the addition of mass detection acts as a complementary technique for impurity analysis.

The ACQUITY Arc System provides increased flexibility for chromatographic separations and maximizes, productivity by accommodating 3.0 µm to 5 µm particles for HPLC methods, while also supporting rapid and efficient UHPLC separations using 2.5 to -2.7 µm particles.[6]

References

1. R M Christie. *Colour Chemistry* Royal Society of Chemistry. Cambridge, 2001.
2. J Garcia-Lavandeira, E Blanco, C Salgado, R Cela. Fast throughput, highly sensitive determination of allergenic disperse dyes in textile products by use of sample composition. (2010) *Talanta*, 82: 261–269.
3. German Institute for Standardisation (DIN). Textiles-Detection-Detection of Dispersed Dyestuffs. DIN 54321:2005.
4. M Gay, J Chang Huang, Q Cai, Q Long Sun. Rapid Screening of 36 Synthetic Dyes using the ACQUITY UPLC H-Class System with the ACQUITY QDa Detector. Waters Technology Brief, no. 720005194en, October 2014.
5. J Cooper and J Marchand. Improving the speed and quantitative performance for the analysis of allergenic and carcinogenic dyes in consumer products. Waters application note no. 720004492en, December 2015.
6. Waters ACQUITY Arc System Brochure, no. 720005393en, June, 2015.

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Improving the Speed and Quantitative Performance for the Analysis of Allergenic and Carcinogenic Dyes in Industrial, Cosmetic, Personal Care and Consumer Products

Jane Cooper[1] and Jérémy Marchand[2]
[1] Waters Corporation, Manchester, UK; [2]University of Nantes, France

APPLICATION BENEFITS

This application note illustrates increased sample throughput for the identification and quantification of allergenic and carcinogenic disperse, acid, direct, and basic dyes in consumer products offering:

- Reduced solvent usage due to reduced run times.
- Improved sensitivity, selectivity, and robustness, compared with existing methodologies.

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

Xevo® TQD

MassLynx® Software

ACQUITY UPLC BEH C_{18} Column

KEY WORDS

Disperse, acid, direct, basic dyes, consumer products, textile, cosmetics, restricted substances, personal care products

INTRODUCTION

Dyes are added to change or add color to a product, with the aim to add appeal and improve sales by making the product more authentically pleasing.

Dyes are used in many products, for example industrial products such adhesive glues and industrial cleaning products; agricultural products such as seed colorants; cosmetics products (for example lipstick and eye shadow); personal care products (for example soaps, hair dye, and wigs); consumer products (for example inks, candles, fabric, paper, and leather); automotive products (for example car washes and polishes).

Originally, all dyes were natural compounds, but gradually a wide range of synthetic dyes were developed that could be produced faster at a lower cost. Synthetic dyes are classified according to how they are used in the dyeing process. Lipophilic disperse dyes are used for dyeing many synthetic fibers, such as polyester, nylon, cellulose acetate, synthetic velvets, and PVC. Whereas, water-soluble dyes, such as anionic acid dyes, cationic basic dyes, and direct dyes have a wide variety of uses on both natural and synthetic fibers. For example, acid dyes can be used on silk, wool, nylon, and modified acrylic fibers; basic dyes can be used on acrylic fibers, wool, silk, and paper; and direct dyes can be used on cotton, paper, leather, wool, silk, and nylon.

Many companies, in order to fulfill their commitment to protect the consumers of their products, their workers, and the community/environment, develop restricted substances lists (RSL). RSL detail both legislated and non-legislated requirements to be upheld in every part of their product supply production chains to reduce or eliminate hazardous substances and processes. In doing so, they also add environmental sustainability value to their products, and ensure that their products are safe and legally compliant. Many potentially hazardous disperse, acid, direct, and basic dyes are detailed in many consumer product suppliers' RSL.

EXPERIMENTAL

Sample description

Textile

- Textile (0.5 g) was cut up and extracted with 20 mL of methanol for 15 min using an ultrasonic bath (50 °C).
- 100 µL of the extract was transferred in an LC vial and diluted with 900 µL of water.

LC conditions

System:	ACQUITY UPLC H-Class
Run time:	7 min
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm
Column temp.:	30 °C
Sample temp.:	10 °C
Mobile phase A:	Water (5 mmol/L ammonium acetate)
Mobile phase B:	Acetonitrile (5 mmol/L ammonium acetate)
Flow rate:	0.6 mL/min
Injection volume:	5 µL

The mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.60	90	10	–
2	0.50	0.60	90	10	6
3	3.00	0.60	5	95	6
4	5.00	0.60	5	95	6
5	5.01	0.60	90	10	6
6	7.00	0.60	90	10	6

Table 1. ACQUITY UPLC H-Class System mobile phase gradient.

MS conditions

Mass spectrometer:	Xevo TQD
Ionization mode:	ESI positive and negative
Capillary voltage:	0.7 kV
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas:	1000 L/h
Cone gas:	20 L/h
Acquisition:	Multiple Reaction Monitoring (MRM)

Examples of both legislated and non-legislated regulations and standards developed by various countries and international organizations with regard to dyes include the following: European Committee for Standardization with regard to toy safety standards (BS EN 71 part 9),[1] Sustainable Textile Production (STeP),[2] European Union Commission Decision (2009/567/EC),[3] the German Food and Commodities law (LFGB § 30), and Cosmetic Directive 1223/2009.[4] All detail many of the potentially sensitizing, carcinogenic, mutagenic, or toxic to reproduction dyes as prohibited.

The standard method for the analysis of disperse dyes in textile products and components is DIN54231,[5] using high performance liquid chromatography (HPLC) or thin layer chromatography (TLC) with either ultraviolet (UV), mass spectrometry (MS), or densitometry detection.

Other methodologies for the analysis of disperse dyes include: electrochromatography with electrospray ionization (ESI) and MS detection,[6] HPLC with: UV/VIS detection,[7] atmospheric pressure chemical ionization (APCI) and MS detection,[8] ESI and MS detection,[9,10] and ion-exchange high-performance liquid chromatography (HPIEC) with MS detection.[11]

This application note, using Waters® ACQUITY UPLC H-Class System coupled with the Xevo TQD, describes the advantages of analyzing disperse, acid, direct, and basic dyes compared to previous methodologies. The results show increased robustness, selectivity, and sensitivity, with reduced run times and associated savings in solvent usage.

MS conditions were optimized, as shown in Table 3, for the analysis of disperse, acid, direct, and basic dyes. CAS numbers, empirical formulas, and structures are displayed in Table 2. The established dyes MRM method, which utilizes fast polarity switching available on the Xevo TQD, is illustrated in Figure 1. This enables the analysis of positive and negative dyes within the same analytical analysis.

Disperse, acid, direct, and basic dyes

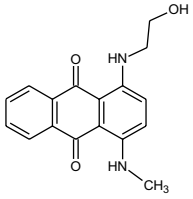
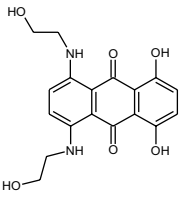
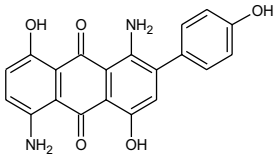
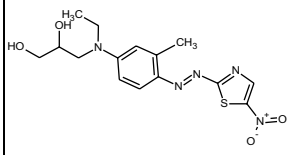
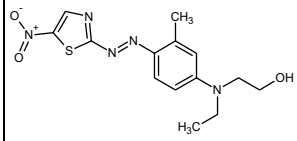
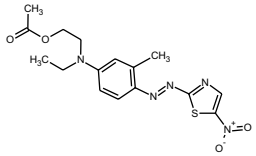
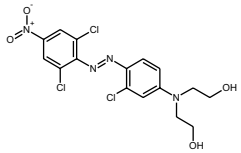
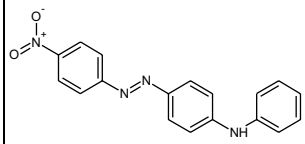
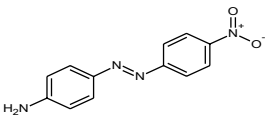
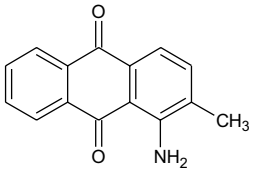
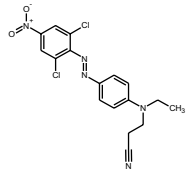
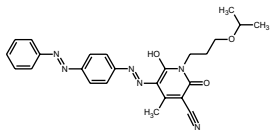
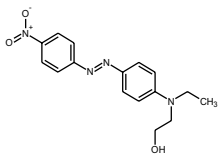
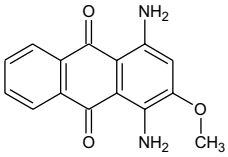
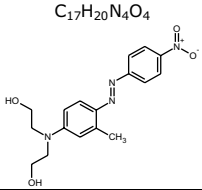
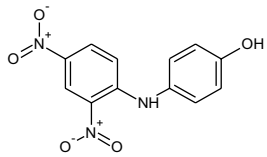
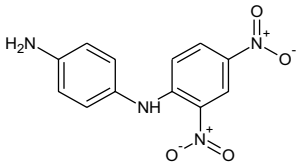
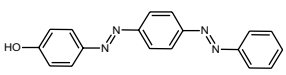
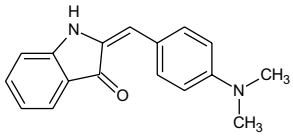
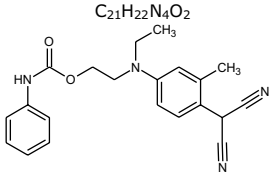
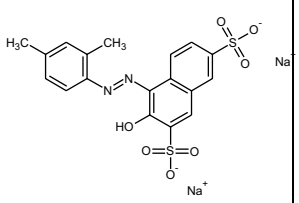
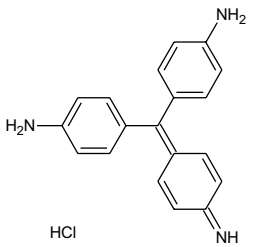
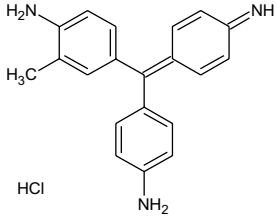
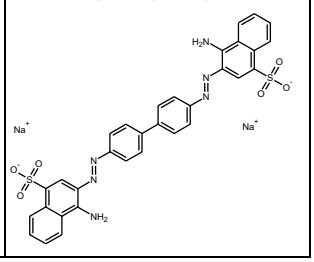
1. **Disperse Blue 3** CAS: 2475-46-9 $C_{17}H_{16}N_2O_3$ 	**2.** **Disperse Blue 7** CAS: 3179-90-6 $C_{18}H_{18}N_2O_6$ 	**3.** **Disperse Blue 35** CAS: 12222-75-2 $C_{20}H_{14}N_2O_5$ 	**4.** **Disperse Blue 102** CAS: 69766-79-6 $C_{15}H_{19}N_5O_4S$ 
5. **Disperse Blue 106** CAS: 68516-81-4 $C_{14}H_{17}N_5OS$ 	**6.** **Disperse Blue 124** CAS: 61951-51-7 $C_{16}H_{19}N_5O_4S$ 	**7.** **Disperse Brown 1** CAS: 23355-64-8 $C_{16}H_{15}Cl_3N_4O_4$ 	**8.** **Disperse Orange 1** CAS: 2581-69-3 $C_{18}H_{14}N_4O_2$ 
9. **Disperse Orange 3** CAS: 730-40-5 $C_{12}H_{10}N_4O_2$ 	**10.** **Disperse Orange 11** CAS: 82-28-0 $C_{15}H_{11}NO_2$ 	**11.** **Disperse Orange 37** CAS: 13301-61-6 $C_{17}H_{15}Cl_2N_5O_2$ 	**12.** **Disperse Orange 149** CAS: 85136-74-9 $C_{25}H_{26}N_6O_3$ 
13. **Disperse Red 1** CAS: 2872-52-8 $C_{16}H_{18}N_4O_3$ 	**14.** **Disperse Red 11** CAS: 2872-48-2 $C_{15}H_{12}N_2O_3$ 	**15.** **Disperse Red 17** CAS: 3179-89-3 $C_{17}H_{20}N_4O_4$ 	**16.** **Disperse Yellow 1** CAS: 119-15-3 $C_{12}H_9N_3O_5$ 
17. **Disperse Yellow 3** CAS: 2832-40-8 $C_{15}H_{15}N_3O_2$ 	**18.** **Disperse Yellow 23** CAS: 6250-23-3 $C_{18}H_{14}N_4O$ 	**19.** **Disperse Yellow 39** CAS: 12236-29-2 $C_{17}H_{16}N_2O$ 	**20.** **Disperse Yellow 49** CAS: 54824-37-2 $C_{21}H_{22}N_4O_2$ 
21. **Acid Red 26** CAS: 3761-53-3 $C_{18}H_{14}N_2Na_2O_7S_2$ 	**22.** **Basic Red 9** CAS: 569-61-9 $C_{19}H_{18}N_3Cl$ 	**23.** **Basic Violet 14** CAS: 632-99-5 $C_{20}H_{20}ClN_3$ 	**24.** **Direct Red 28** CAS: 573-58-0 $C_{32}H_{22}N_6Na_2O_6S_2$ 

Table 2. Disperse, acid, direct, and basic dyes, associated CAS numbers, empirical formulas, and structures.

No	Chemical substance	Retention time (min)	ESI (+/-)	Cone voltage (V)	Transition	Collision energy
1	Disperse Blue 3	2.41	+	45	297.0 > 235.1	33
					297.0 > 252.0 *	21
2	Disperse Blue 7	2.26	+	50	359.0 > 283.0 *	32
					359.0 > 314.0	20
3	Disperse Blue 35	2.97	+	36	285.0 > 185.0	12
					285.0 > 270.0*	28
4	Disperse Blue 102	2.53	+	42	366.0 > 147.0	31
					366.0 > 208.1*	18
5	Disperse Blue 106	2.71	+	42	336.0 > 147.0	35
					336.0 > 178.0*	17
6	Disperse Blue 124	3.04	+	39	378.1 > 160.1	23
					278.0 > 220.1*	16
7	Disperse Brown 1	2.84	+	53	433.0 > 197.1*	31
					433.0 > 357.0	37
8	Disperse Orange 1	3.36	+	49	319.0 > 122.0*	22
					319.0 > 169.0	26
9	Disperse Orange 3	2.77	+	45	243.0 > 92.0	22
					243.0 > 122.0*	18
10	Disperse Orange 11	2.80	+	53	238.0 > 165.0*	30
					238.0 > 223.0	25
11	Disperse Orange 37	3.27	+	50	392.0 > 133.0*	38
					392.0 > 350.9	22
12	Disperse Orange 149	3.60	-	69	457.1 > 121.0*	52
					457.1 > 266.0	33
13	Disperse Red 1	2.91	+	51	315.1 > 134.0*	25
					315.1 > 284.1	23
14	Disperse Red 11	2.40	+	51	268.0 > 225.0*	28
					268.0 > 253.0	21
15	Disperse Red 17	2.64	+	53	345.1 > 164.1*	26
					345.1 > 269.1	28
16	Disperse Yellow 1	2.57	-	32	274.0 > 166.0*	12
					274.0 > 226.0	15
17	Disperse Yellow 3	2.80	-	37	268.0 > 134.0*	18
					368.0 > 253.0	18
18	Disperse Yellow 23	3.37	+	46	303.1 > 105.0*	21
					303.1 > 181.0	17
19	Disperse Yellow 39	2.83	+	55	291.0 > 130.0*	29
					291.0 > 245.1	28
20	Disperse Yellow 49	3.02	-	22	373.1 > 168.0*	27
					373.1 > 209.1	21
21	Acid Red 26	1.80	+	47	437.0 > 121.1*	25
					437.0 > 355.1	19
22	Basic Red 9	2.01	+	60	288.2 > 195.1*	33
					288.2 > 271.1	35
23	Basic Violet 14	2.12	+	68	302.1 > 195.1	35
					302.1 > 209.1*	32
24	Direct Red 28	2.02	-	81	325.0 > 81.0	27
					325.0 > 152.0*	23

*Table 3. Disperse, acid, direct, and basic dyes, expected retention times, ionization mode, cone voltages, MRM transitions, and associated collision energy values (*refer to the quantification transition).*

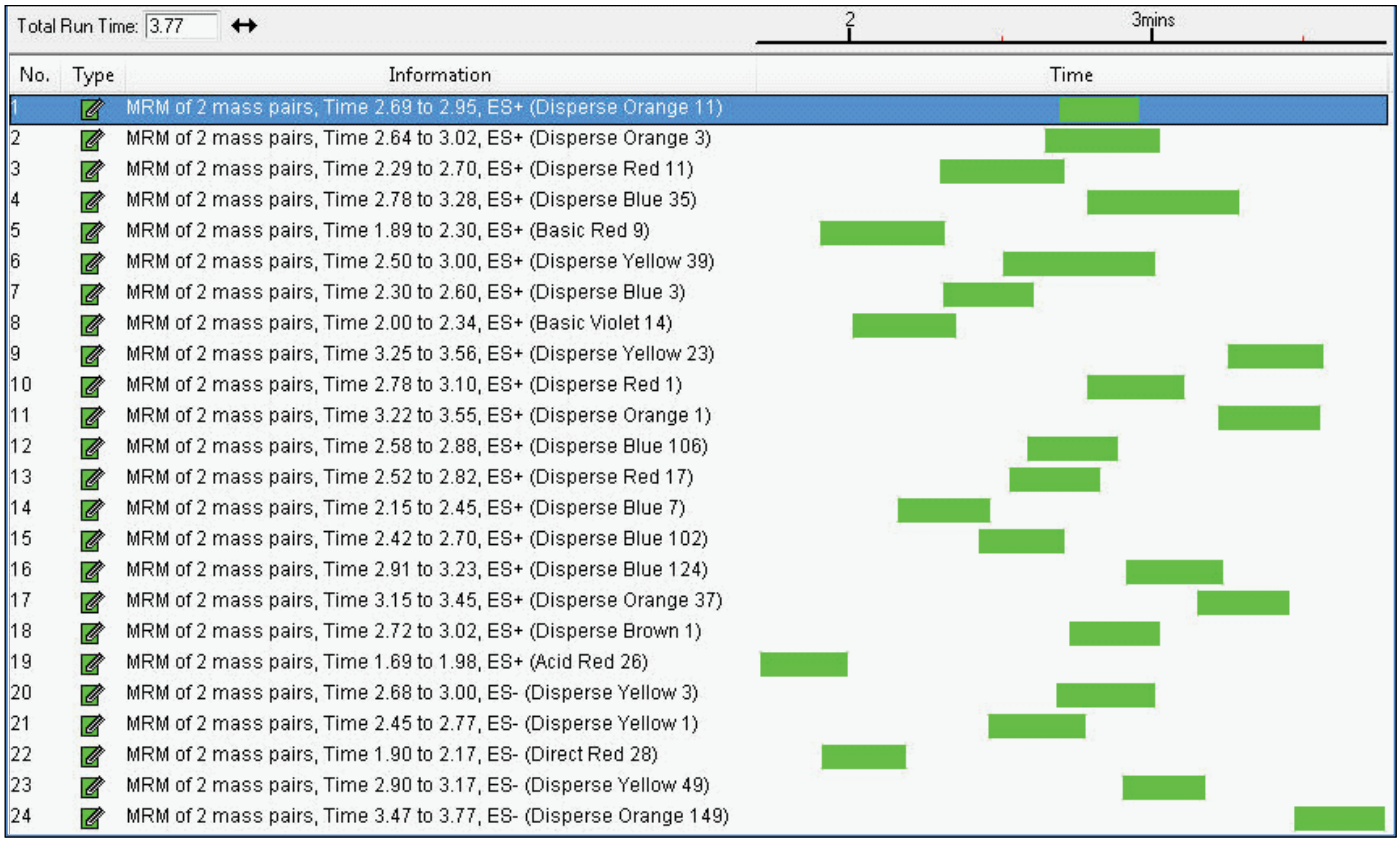


Figure 1. MRM method for 24 disperse, acid, direct, and basic dyes.

Instrument control, data acquisition, and results processing

MassLynx Software was used for data acquisition, and control of the ACQUITY UPLC H-Class System and the Xevo TQD. Data quantification was achieved using the TargetLynx™ Application Manager.

RESULTS AND DISCUSSION

The analysis of 24 disperse, acid, direct, and basic dyes was achieved using Waters' Xevo TQD in MRM mode with ESI ionization, coupled with the ACQUITY UPLC H-Class System.

Optimum MRM conditions were developed and, initially, HPLC conditions based on the work performed by Qiang *et al.*[7] (mobile phase, column, and gradient) were implemented. The method migration from HPLC to UPLC was aided by using tools developed by Waters including the following: the Waters Column Selectivity Chart[12-13] to aid the selection of a suitable UPLC column and the ACQUITY UPLC Column Calculator[13] to aid the development of UPLC gradient and flow. The optimized UPLC conditions resulted in the elution of all compounds within a seven minute run.

The fast cycle and polarity switching times of the Xevo TQD enable the UPLC narrow peaks to be efficiently resolved. A comparison between HPLC and UPLC chromatograms is shown in Figure 2, illustrating improvements in sensitivity and sample throughput.

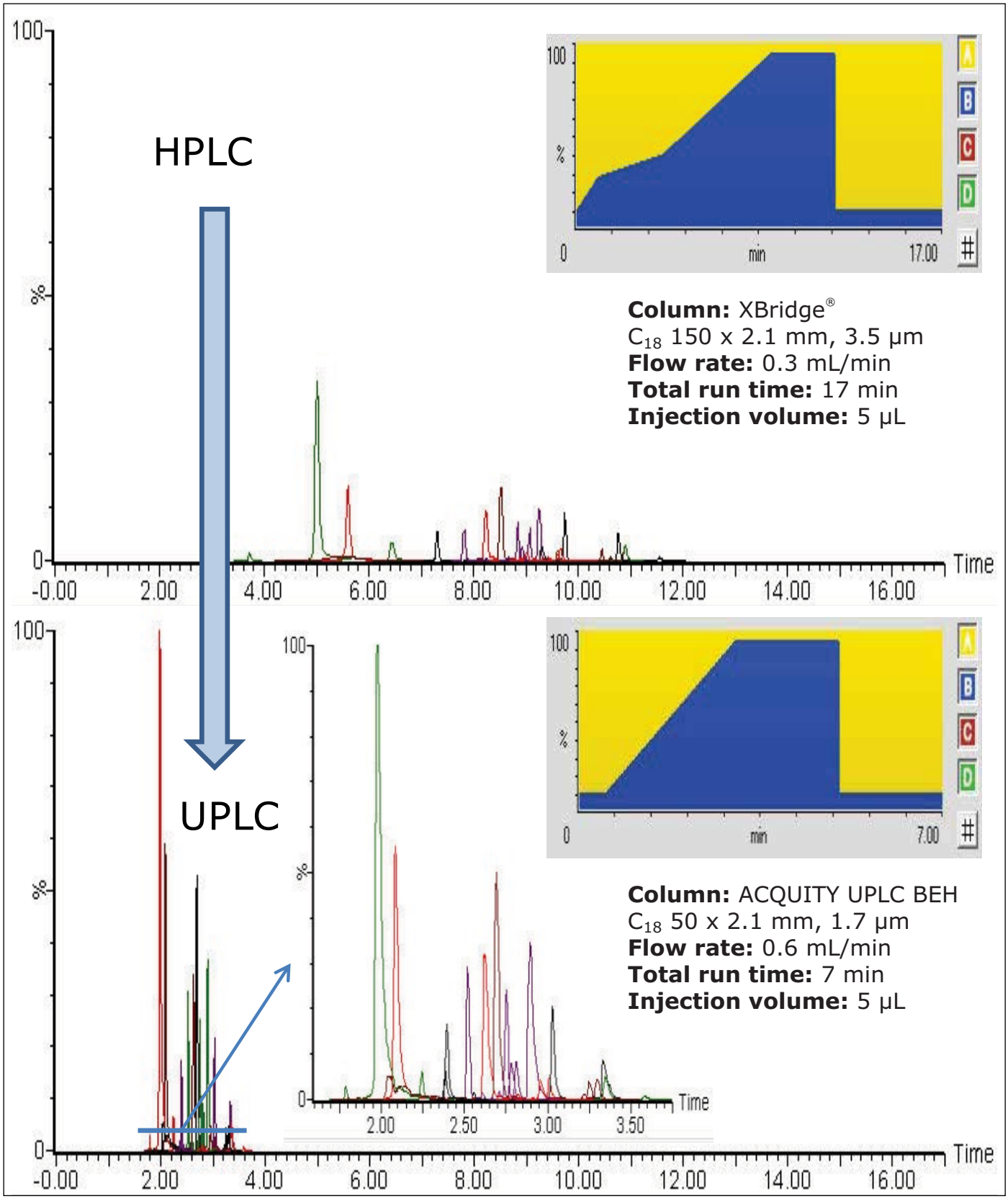
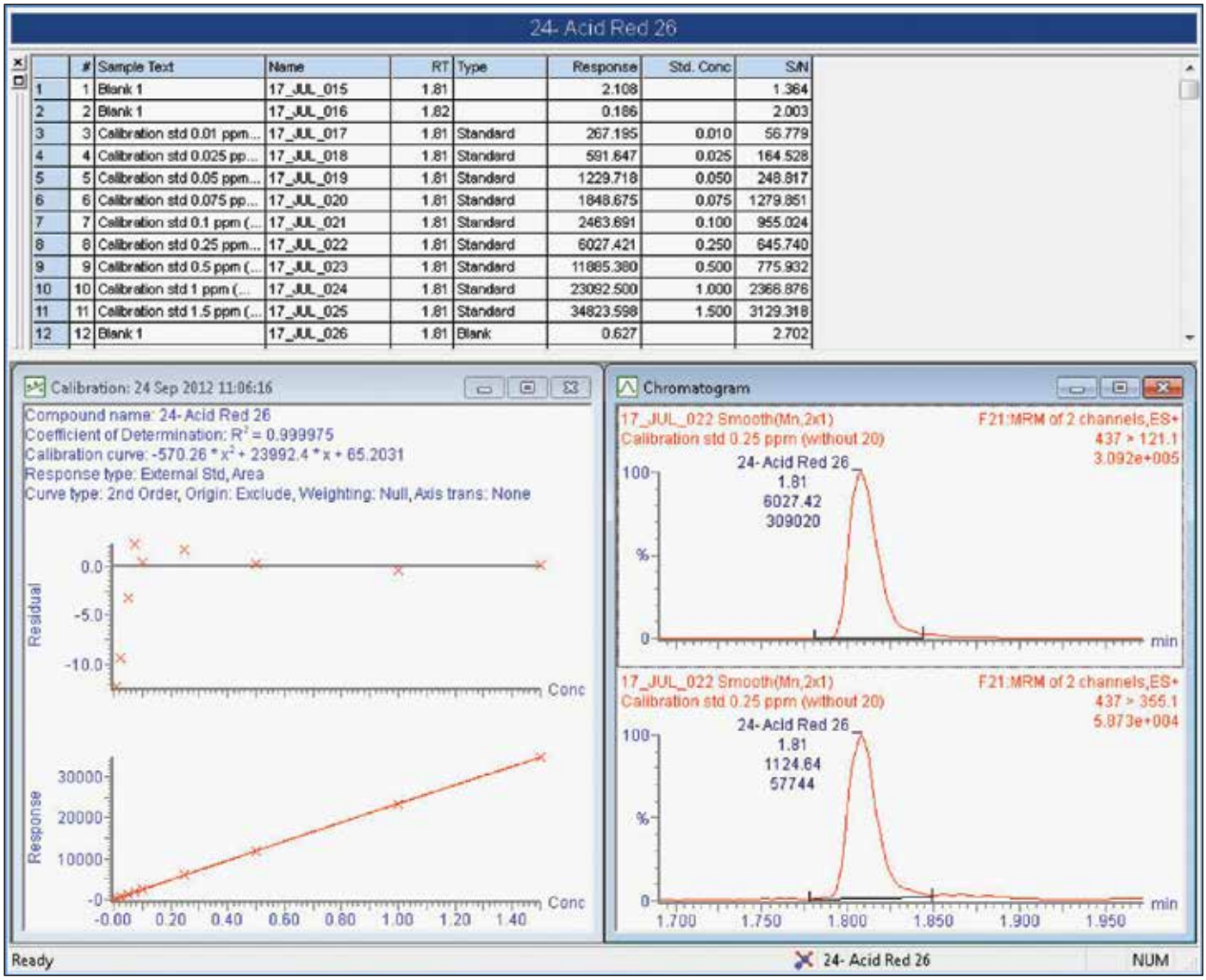


Figure 2. HPLC and UPLC overlaid 1 ppm chromatograms, mobile phase A: water (5 mmol/L ammonium acetate), and mobile phase B: acetonitrile (5 mmol/L ammonium acetate).

Mixed calibration standards, ranging from 0.01 to 1.5 µg/mL, were prepared and analyzed for all of the compounds considered (equivalent range of 4 to 600 µg/g in textile samples). The TargetLynx Quantify results for acid red 26 are shown in Figure 3, and the MRM chromatograms for each compound are shown in Figure 4.



	#	Sample Text	Name	RT	Type	Response	Std. Conc	S/N
1	1	Blank 1	17_JUL_015	1.81		2.108		1.364
2	2	Blank 1	17_JUL_016	1.82		0.186		2.003
3	3	Calibration std 0.01 ppm...	17_JUL_017	1.81	Standard	267.195	0.010	56.779
4	4	Calibration std 0.025 pp...	17_JUL_018	1.81	Standard	591.647	0.025	164.528
5	5	Calibration std 0.05 ppm...	17_JUL_019	1.81	Standard	1229.718	0.050	248.817
6	6	Calibration std 0.075 pp...	17_JUL_020	1.81	Standard	1848.675	0.075	1279.851
7	7	Calibration std 0.1 ppm (...	17_JUL_021	1.81	Standard	2463.691	0.100	955.024
8	8	Calibration std 0.25 ppm...	17_JUL_022	1.81	Standard	6027.421	0.250	645.740
9	9	Calibration std 0.5 ppm (...	17_JUL_023	1.81	Standard	11885.380	0.500	775.932
10	10	Calibration std 1 ppm (...	17_JUL_024	1.81	Standard	23092.500	1.000	2366.876
11	11	Calibration std 1.5 ppm (...	17_JUL_025	1.81	Standard	34823.598	1.500	3129.318
12	12	Blank 1	17_JUL_026	1.81	Blank	0.627		2.702

Figure 3. TargetLynx Quantify results browser showing the calibration quantification results, calibration curve, and example MRM chromatogram for acid red 26.

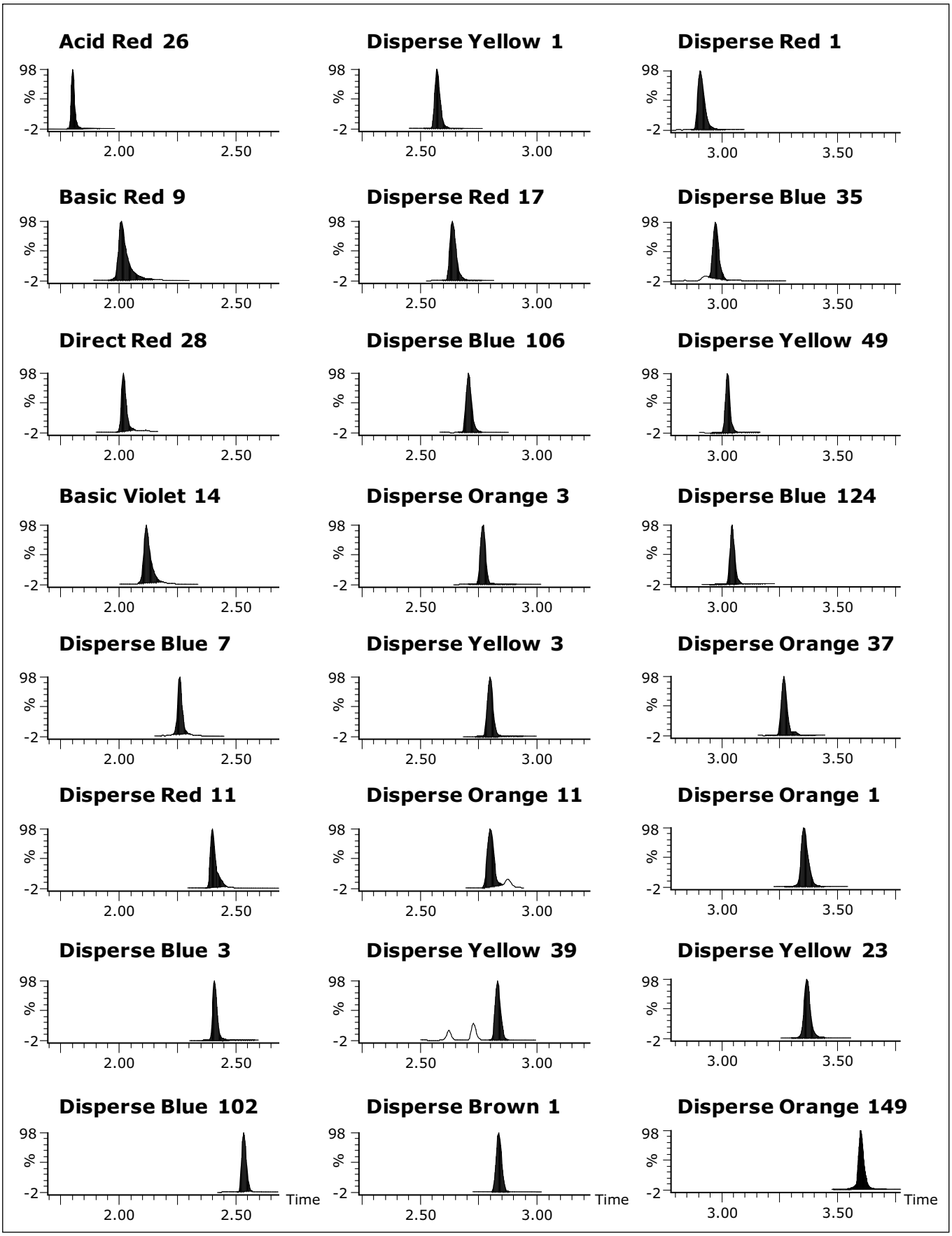


Figure 4. MRM chromatograms for disperse, acid, direct, and basic dyes in a mixed 0.5 µg/mL calibration standard (equivalent to 200 µg/g in textile samples).

Textile analysis

The MRM mass detection method, shown in Figure 1, was used after appropriate sample preparation to quantify for dyes.

Using the extraction protocol (based on DIN 54231)[5] and the instrument parameters as detailed, the results obtained for the analysis of synthetic textile samples spiked at 75 and 30 µg/g are shown in Table 4. Many laboratories that base their extraction protocol for disperse dyes on DIN 54231,[5] accept 75 µg/g as the practical detection limit. Recoveries were obtained by comparing extracted spiked textile samples with calibration standards.

Dye	Sample	Replicate injection results (µg/g)			Average recovery (blank corrected) %	RSD (%)
		1	2	3		
Disperse Brown 1	Blank	ND	ND	ND	-	-
	75 µg/g	67.7	71.6	74.8	95.1	5.0
	30 µg/g	27.7	27.2	27.2	91.2	1.1
Disperse Red 1	Blank	ND	ND	ND	-	-
	75 µg/g	75.3	75.0	78.8	102	2.8
	30 µg/g	33.2	31.8	33.7	110	3.3
Disperse Yellow 1	Blank	ND	ND	ND	-	-
	75 µg/g	77.1	80.9	82.2	107	3.3
	30 µg/g	28.0	30.4	29.5	97.7	4.1
Disperse Yellow 39	Blank	0.28	0.36	0.40	-	-
	75 µg/g	74.0	80.8	81.6	105	5.4
	30 µg/g	30.3	30.4	31.2	101	1.6
Disperse Yellow 49	Blank	ND	ND	ND	-	-
	75 µg/g	71.2	72.6	73.8	96.7	1.8
	30 µg/g	27.3	27.0	27.7	91.1	1.3

Table 4. Textile samples spiked with selected disperse dyes recovery data. Results obtained using mass spectrometric detection and quantified against mixed calibration standards. ND = not detected.

Efficient recoveries were obtained, ranging between 91% and 110% for the three replicates.

Additional benefits over previous methodology include improved selectivity and sensitivity for the analysis of dyes using the ACQUITY UPLC H-Class System coupled with the Xevo TQD with reduced run times, and associated savings in solvents.

CONCLUSIONS

By utilizing the ACQUITY UPLC H-Class System coupled with the Xevo TQD, a fast, selective, and sensitive method was developed for the analysis of disperse, acid, direct, and basic dyes.

Rapid polarity switching technologies, available on the Xevo TQD, enabled UPLC analysis of positive and negative dyes from a single injection.

The described approach offers the following benefits when compared with standard methodology:

- Business benefits of using UPLC analysis, when comparing HPLC/UV to UPLC/MS analysis, include a greater than five times increase in sample throughput and more than an 86% reduction in solvent usage.
- Enhanced sensitivity and selectivity resulting in improved confidence in the identification and quantification offered by the ACQUITY UPLC H-Class System coupled with the Xevo TQD.
- Fast method migration from HPLC to UPLC aided by the use of tools developed by Waters including the following: the Column Selectivity Chart used to aid the selection of a suitable UPLC column, and the ACQUITY UPLC Column Calculator used to aid the development of UPLC conditions.

References

1. BS EN 71-9:2005+A1:2007 Safety of toys. Organic chemical compounds. Requirements.
2. Sustainable Textile Production (STeP). OEKO-TEX Association. [cited 2015 October 14]. Available from: https://www.oeko-tex.com/en/manufacturers/concept/sustainable_textile_production_step/step.xhtml
3. The Commission of the European Communities. Commission Decision of 9 July 2009 establishing the ecological criteria for the award of the Community Ecolabel for textile products (2009/567/EC). Official Journal of the European Union. L 197: 70–86, 9th Jul 2009. [cited 2012 September 20]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:197:0070:0086:EN:PDF
4. The European Parliament and the Council of the European Union. Regulations (EC) No 1223/2009 of The European Parliament and of the Council of 30 November 2009 on cosmetic products. Official Journal of the European Union. L 342: 59-209, 30th November 2009. [cited 2015 August 25]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF
5. German Institute for Standardization (DIN). Textiles – Detection of dispersed dyestuffs. DIN54231:2005.
6. Lord GA, Gordon DB, Tetler LW, Carr CM. Electrochromatography-electrospray mass spectrometry of textile dyes. *J Chromatography A*. 1995; 700:27–33.
7. Qiang M, Hua B, Qing Z, Wei M, *et al.* Determination of carcinogenic and allergenic dyestuffs in toys by LC coupled to UV/Vis spectrometry and tandem mass spectrometry. *Chromatographia*. 2010; 72:85–93.
8. Morgan S, Vann B, Baguley B, Stefan A. Advances in discrimination of dyed textile fibers using capillary electrophoresis/mass spectrometry. Proceedings of the FBI Trace Evidence Symposium, Clearwater, FL, 15 August 2007. [cited 2012 September 20]. Available from: http://projects.nfstc.org/trace/docs/final/morgan_dyed_textiles_revised.pdf.
9. Ràfols C, Barceló D. Determination of mono- and disulphonated azo dyes by liquid chromatography–atmospheric pressure ionization mass spectrometry. *J Chromatography A*. 1997; 777:177–192.
10. Holcĭapek M, Jandera P, Prĭikryl J. Analysis of sulphonated dyes and intermediates by electrospray mass spectrometry. *Dyes and Pigments*. 1999; 43:127–137.
11. Socher G, Nussbaum R, Rissler K, Lankmayr E. Analysis of sulfonated compounds by ion-exchange high performance liquid chromatography-mass spectrometry. *J Chromatography A*. 2001; 912:53–60.
12. Waters reversed-phase column selectivity chart. [cited 2012 September 20]. Available from: http://www.waters.com/waters/promotionDetail.htm?id=10048475
13. Craven K. HPLC to UPLC Method Migration Using Acrylate Analysis as a Model. Application Note 720004105en. 2011 Sept.

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