Clinical and Toxicology Applications

Session 150-5 • Rm S502b • 2:35 PM (Slot #6)
Quantitative Analysis with a New Physiological Amino Acid Analysis Method
Paula Hong, Waters Corporation

Abstract:
Amino acid analysis is an important tool in the study of a number of physiological processes. Changes in the concentrations of specific amino acids can result from various modifications at specific points in metabolic pathways. These changes may be due to the influence of nutrition on metabolism, as well as, environmental and genetic disturbances of metabolism. As more of these conditions have been identified, the need to analyze more samples has become significant. Improving throughput is a function of both reducing run time and improving method robustness to reduce the need for re-analyses. In addition, it has become apparent that understanding these processes require reliable analysis at lower concentrations. Current ion exchange methods are time-consuming, often require method adjustment, and do not reach the required sensitivity. We have previously introduced an application solution, for research use only, that addresses these concerns. This approach includes a well-characterized amino acid derivatization (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate), a tested UltraPerformance LC® (UPLC®) method for analysis of the derivatized amino acids, and compliance-ready software for acquisition and reporting. We will present studies demonstrating the quantitative analysis with this solution. These studies include the linearity and sensitivity of each of the commonly measured amino acids in a physiological profile. Accuracy and precision for both standards and biological samples will be examined. Samples in these biological matrices will also be compared to existing standard methods. These quantitative studies will show the reliability and robustness of this solution for the analysis physiological amino acids.

Organized Contributed Session
ACS Division of Analytical Chemistry: Understanding Chromatography with Sub-2 μm Particles

Session 320-2 • Rm S404bc • 8:50 AM (Slot #2)
Achieving Maximum Performance with Sub-2 μm Particles
Kenneth Fountain, Waters Corporation

Abstract:
The use of sub-2 μm particle LC columns designed for fast and/or high resolution separations has quickly gained popularity and acceptance in many chromatographic laboratories, due to the increased speed, resolution, and sensitivity over traditional HPLC. A common misconception is that using sub-2 μm particle columns with conventional chromatographic systems can produce high efficiency separations without the need for UHPLC instrumentation. However, sub-2 μm particle columns are more susceptible to extra-column band spreading. Additionally, operating sub-2 μm particle columns at their optimum linear velocities generates backpressures that are not achievable with traditional HPLC instrumentation. Therefore, sub-optimal flow rates are often used, which deteriorates separation integrity due to the influence of analyte diffusion. The effects of system volume, operating pressure, and separation temperature were investigated for sub 2-μm particle columns. The system volume contribution to band spreading on conventional HPLC instrumentation was found to be the primary reason for sub-optimal performance of sub 2-μm columns. In addition, traditional instrumentation was found to be incapable of operating at the backpressures necessary for using sub 2-μm particles at their optimal flow rates, resulting in over a 40 % reduction in measured efficiency. Elevated column temperature was investigated to minimize column backpressure. Results demonstrate a decrease in backpressure and retention time, as well as a 12 % decrease in column efficiency at the same flow rate. In order to maintain column efficiency at elevated temperature, flow rate was increased, resulting in constant column pressure at the optimal linear velocity, independent of column temperature.
A Simple RP and HILIC LC/MS/MS Strategy for Retaining Hydrophobic and Polar Molecules
Erich Grumbach, Waters Corporation

Abstract:
When faced with the task of analyzing a number of compounds encompassing a broad range of polarity, few options exist that will result in adequate retention of polar species without excessive retention of the less polar components. In drug discovery, a large number of candidates need be screened, often with little or no previous characterization of their chemical properties. Many polar candidates are often overlooked due to their elution in the void space of the column, while hydrophobic species may be permanently bound to the stationary phase. A simple generic LC/MS/MS strategy for in vitro or in vivo samples is discussed that imparts exceptional retention of polar actives and metabolites, while affording balanced retention of hydrophobic molecules. Additionally, hydrophilic interaction chromatography (HILIC) is discussed as an attractive alternative to RP that utilizes high organic HILIC mobile phases to provide increased sample throughput and exceptional mass spectrometry response to improve the reliability, sensitivity and throughput of quantitative bioanalytical assays.

A User-friendly Scalable Modular HPLC System for the Development Laboratory and QC Facility
Patricia McConville, Waters Corporation

Abstract:
High-performance liquid chromatography (HPLC) has evolved into one of the most powerful and versatile tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC has been applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals. Within these various industries, there is a need for a rugged, easy-to-use scalable HPLC system which also has a simplified software interface in the local language. Given the broad range of samples that HPLC is applied to, the system must be capable of supporting both isocratic and gradient separations and accommodate multiple modes of detection, including photodiode array. This session employs a number of separation examples from several industries to illustrate the capabilities and performance of a new modular HPLC software system for use where the complexity of the analytical problem does not justify the purchase of more advanced solutions.
Symposia
High Speed Liquid Chromatography

Session 1760-1 • Rm S404d • 8:35 AM (Slot # 2)
The Interrelationship Between Pressure and Column Performance
Uwe D. Neue, Waters Corporation

Abstract:
The use of very high pressures has put new options on the table. For small molecules, short columns permit very fast separations at these pressures, while long columns at elevated temperature create high separation performance in a short time. There continue to be discussions around the best particle size or particle form. We will address this issue in this presentation. In addition, the use of high pressure for the separation of large molecules (proteins and peptides) has become of interest. One can apply the same tools as used for small molecules to understand the optimal operating conditions for large molecules in reversed-phase chromatography. Examples from this application area will be shown.

Organized Contributed Session
ACS Division of Analytical Chemistry Multi-residue Pesticide Analysis for Food Testing

Session 1810-3 • Rm S501bc • 9:10 AM (Slot #3)
Comparison of Derivatization GC/MS(MS) with LC/MS(MS) for the Determination of Acidic Pesticides in QuEChErS™ Extracts of Fruits and Vegetables
Michael S. Young, Waters Corporation

Abstract:
Recently, a rapid solvent and salt-out partition procedure has become popular for pre-treatment of fruits and vegetables prior to dispersion or pass-through SPE cleanup using an aminosilica sorbent such as PSA. These procedures, known collectively as QuEChErS method, are designed to produce extracts for GC/MS and LC/MS analysis. The procedure has been demonstrated for a wide range of basic and neutral pesticides, but PSA cleanup is not compatible with analysis of acidic compounds. We have previously discussed cleanup procedures useful for UPLC/MS determination of acidic pesticides in such extracts. However, since GC/MS analysis is still commonly utilized for these analytes, this presentation will discuss optimized cleanup protocols suitable for both GC/MS and LC/MS analysis. In this context, we will compare the two instrumental chromatography/mass spectrometry techniques.
Scientific Presentations

Product Status / Process Stream Measurements

Session 1910-4 • Rm S505a • 9:30 AM (Slot #4)

Utilizing UPLC for the At-Line and On-Line Analysis of In-Process Samples
Tanya Jenkins, Waters Corporation

Abstract:
Process Analytical Technology or PAT is an important part of the overall manufacturing process to ensure final product quality and improve product yields. PAT involves taking measurements throughout the production process to verify the quality of in-process batches and understand the critical steps of the process. Typically, these steps are reaction monitoring and product purification which are assessed by spectroscopic sensors such as near-infrared spectroscopy (NIR) or Raman spectroscopy. These techniques have the ability to provide real-time information about processes, but they lack the ability to simultaneously monitor multiple components at different levels. The performance of these sensors is typically benchmarked against a reference standard, which in many instances is high performance liquid chromatography (HPLC) because it is a more selective, sensitive, and linear technique. HPLC is the most widely used technique in pharmaceutical QC laboratories, however, the relatively long runtimes and complex system architecture have prevented it from being routinely used for at-line or on-line analysis. The introduction of UltraPerformance LC (UPLC) makes it possible to achieve near real-time analysis for in-process samples. In this session will discuss implementing UPLC for on-line reaction monitoring and the monitoring of the effluent from a process purification column. The fast runtimes (less than 2 minutes) yield an excellent map of the reaction or process column chromatography. UPLC also provides the ability to simultaneously and repeatedly quantitate both high and low level components (below 0.1% of the major component). At-line applications, requiring automated sample preparation and a simple walk-up interface for process technicians, are discussed.

Organized Contributed Session
Achievements and Challenges in Mass Spectrometry

Session 2100-5 • Rm S402b • 03:05 PM (Slot #6)

UPLC-MS/MS Determination of ppt Levels of Perfluorinated Acidic Compounds in the Environment:
Sample Preparation and Instrumental Considerations
Michael S. Young, Waters Corporation

Abstract:
Perfluorinated acids and related compounds (PFCs) such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been identified as persistent organic pollutants (POPs) and may be ubiquitous in the environment. Because PFCs may be toxic and may have bioaccumulative properties, there is growing interest in the development of analytical methods for PFCs in water, biota, soil, and food. The analytical requirements for this analysis are particularly challenging because very low detection limits (under 1 ng/L for aqueous samples) are required. In this presentation, we will discuss sample preparation issues and instrumental requirements for UPLC-MS determination of PFCs in environmental samples. Sample preparation topics will include the choice of solid-phase extraction (SPE) sorbents and protocols used for both solid and aqueous samples. Instrumental topics will include the optimization of UPLC and MS/MS parameters for the low detection limits required for these analyses. A primary topic will be reduction or elimination of background PFC contamination resulting from the mobile-phase or other external or internal sources. The elimination of this background contamination can result in ten-fold or greater improvement in detection limits.
Organized Contributed Session
ACS Division of Analytical Chemistry Analysis of Proteins, Peptides, Amino Acids, and Nucleic Acids by LC and LC/MS

Session 2440-7 • Rm S404d • 10:45 AM (Slot #8)
A Comprehensive Approach to Developing Selective and Sensitive Bioanalytical Methods for Peptide Biopharmaceuticals in Human Plasma
Erin E. Chambers, Waters Corporation

Abstract:
The evaluation of a candidate medicine requires the accurate determination of the pharmacokinetics and metabolic fate of the active ingredient in human and animal species. This testing requires a specific and selective methodology to allow for high sensitivity analyte detection in biological matrices with high throughput. Recent changes in the regulations, such as incurred sample reanalysis and assessment of matrix effects, place an extra burden analytical methodology. We will show how, with the use of a mixed mode ion-exchange solid-phase process coupled with a high resolution LC system can dramatically reduce sample variability due to matrix effects. Highly sensitive and selective methods for four peptide therapeutics in human plasma were developed using this approach. The development of bioanalytical methods for peptides is complicated by several factors. Peptides are zwitterionic, making their retention behavior on SPE sorbents difficult to predict and increasing method development times. Because of their size and charge state distribution, sensitivity by mass spectrometry may be lower for biomolecules than typical small molecules, necessitating concentration. A simple SPE screening consisting of one protocol and two mixed-mode sorbents was employed to simplify SPE method development. 300 μL of human plasma was loaded onto the SPE device, eluted in 50 μL, diluted with 0.2% TFA in water to improve peak shape, and directly injected for analysis. Absolute matrix effects for all compounds were determined to be < 10% and extraction recovery > 85%. SPE was performed using the μ Elution format to minimize sample volume used and to concentrate analytes, necessary to reach the desired limits of detection, without evaporation and reconstitution of the final eluates. This improves detection limits for biopharmaceuticals and eliminates potential losses during evaporation.

Symposia
Global Issues Facing the Food Industry

Session 2810-5 • Rm S401d • 3:55 PM (Slot #6)
Regulating Food Safety for a Global Marketplace
Paul Young, Joe Romano, Waters Corporation

Abstract:
As a result of globalization in food markets, the value of food commodity exports are increasing significantly. FAO figures indicate that agricultural exports increased by almost 60% between 2000-2005 to approach $700 Billion. At the same time, increased emphasis has been placed on the importance of food safety by consumers, producers, and legislative bodies worldwide. This has resulted in the need for more comprehensive testing with greater sensitivity and also in strict legislative criteria defining the suitability of confirmatory analytical techniques. This is particularly evident in the fields of pesticide and veterinary drug residue analysis. There is a demand therefore to create analytical techniques that are capable of simultaneously detecting and unequivocally confirming the presence, of increasing numbers agricultural chemicals in ever shorter periods of time. Multi-residue confirmatory analyses pose a number of challenges in relation to chromatographic resolution and mass spec. instrument duty cycle. This presentation will discuss the application of UPLC to increase throughput combined with recent advances in MS technologies for food safety analysis.