OPEN HOUSE & POSTER SESSION

September 17, 2014
6 pm - 8 pm

Bethesda North Marriott Hotel & Conference Center
5701 Marinelli Road, North Bethesda, MD

http://wcdg.squarespace.com

Table of Contents

Dear Colleagues,

The Washington Chromatography Discussion Group (WCDG) thanks you for participating in the fourth annual WCDG Open House & Poster Session. On behalf of the group, I hope that you enjoy the science and networking opportunities that an event like this provides. The WCDG is composed of several hundred enthusiastic separation scientists in the Washington DC area with interests in separations techniques such as LC, GC, and CE. Our membership is composed of industry, government, and academic professionals with a goal of expanding our understanding in chromatography and its applications.

As your host for this event, I wish to welcome you to our Open House & Poster Session and to thank you for your participation.

Sincerely,

Jonathan Edelman
WCDG President

Characterization of star polystyrenes and their linear analogues by non-aqueous asymmetric flow field-flow fractionation

Leena Pitkanen
National Institute of Standards & Technology
Gaithersburg, MD
leena.pitkanen@nist.gov

Coauthors: André M. Striegel

Abstract:
Asymmetric flow field-flow fractionation (AsFlFFF) is an elution-based technique for analysis of macromolecules, colloids, and particles. Separation takes place in an open channel, based on differences in the diffusion coefficients of analytes. Because AsFlFFF is a relatively gentle separation technique in comparison with conventional chromatographic techniques, it has found favor in the analysis of water-soluble macromolecules, molecular assemblies, and even entire cells [1]. At the same time, only very few reports can be found describing AsFlFFF work conducted in organic solvents. Here, we present just such application of AsFlFFF, in tetrahydrofuran, for characterization of narrowly distributed polystyrene stars and their linear analogues. A cross-flow gradient was employed to achieve separation of individual polystyrenes in mixtures. Multiple-detection allowed determination of molar mass and size for each separated polystyrene within a single run and without reliance on calibrants or prior knowledge of channel dimensions.

[1] G. Yohannes, M. Jussila, K. Hartonen and M.-L. Riekkola, J. Chromatogr. A, 2011, 1218, 4104-4116.

Efficient Methods Development Combining Simultaneous Mass and UV Detection with Flexible Software for Data Analysis and Mobile Phase Formulation

Annie Landicho
Waters Corporation
Milford, MA
annie_landicho@waters.com

Coauthors: Paula Hong, Patricia R. McConville

Abstract:
Methods development for reversed-phase liquid chromatographic (RPLC) separations typically requires many time-consuming steps, including manual preparation and pH adjustment of mobile phases, as well as extensive data processing. Also a single detection technique provides insufficient information for missed peaks and co-elutions: Isobaric compounds can be difficult to distinguish with a mass detector alone, while peak identification with UV is not possible for compounds that lack a chromophore. To address some of these challenges, multiple detectors can be used for analysis of a single sample with each detection technique dependent on a different physical or chemical property of the molecule.

In this presentation, we will describe an efficient methods development approach that combines both dual detection and automated software for mobile formulation. This strategy will evaluate a variety of factors including mobile phase pH, organic solvent, temperature, stationary phase and physical parameters for a systematic method development approach. Manipulation of mobile phase pH will be demonstrated through the use of flexible software, while a comprehensive software for simultaneous analysis of both mass and UV spectral data will allow for simplified data processing. The effect of these physical and chemical parameters will be illustrated with both mass and UV spectral data for peak identification. The benefits of peak tracking with combination of mass and UV spectra will be demonstrated for a variety of compounds including natural products and pharmaceuticals.

Development of Buffer Systems Providing pH Control for LC-UV-MS Method Development

Annie Landicho
Waters Corporation
Milford, MA
annie_landicho@waters.com

Coauthors: Thomas E. Wheat, Aparna Chavali, Patricia R. McConville

Abstract:
Reversed-phase method development typically includes screening and optimizing chromatographic parameters such as gradient slope, flow rate, temperature, column length, organic solvent and modifier concentration, and so on. The adjustment of pH can serve as a useful addition to these screening tools. Common modern practice screens high and low pH regimes far from the analyte pK to protonate or to deprotonate completely the molecules. This effective procedure eliminates the acidic and basic functional groups from contribution to the separation selectivity. Near the pK, however, relatively small changes in pH can make the molecules behave as though partially charged, reflecting the molecular structure near the ionizable group. This leads to significant alteration in the selectivity between similar compounds and those with common functional groups. It is cumbersome to exploit this principle in a method development experiment because of the number of mobile phases to be prepared. We have developed an efficient automation of such experiments by integrating the blending of stock buffers by the liquid chromatography system to deliver mobile phases of different pH. To extend this utility to all the compounds in a sample often requires selection of a different buffer system to cover a different pK range. It would be useful to have a protocol which can be applied to unknown compounds consisting of different functional groups. We describe here a single general buffer system that gives stable pH control over the range from pH 2 to pH 11. It is compatible with multiple detection modes including electrospray MS and low wavelength UV. It can be used for different applications such as impurity profiling, stability testing and can be used with unknown compounds that have a mix of analytes sensitive to different ranges in the pH spectrum.

Pegylated Protein Analysis by Size-Exclusion and Reversed Phase UHPLC

Laura Mulderig
Waters Corporation
Newark, DE
laura_mulderig@waters.com

Coauthors: William Warren, Stephan Koza, Kenneth Fountain

Abstract:
Size-Exclusion UHPLC (SE-UHPLC) can provide rapid analysis of the products and unreacted components of a protein PEGylation reaction if the Rh values for the non-PEGylated protein,activated PEG (aPEG), and conjugate are sufficiently different. Based on predictions of the Rh, radius of hydration, values for combinations of protein and PEG molecular weights, in many circumstances SE-UPLC cannot provide the necessary analytical separation of all three components. This was indeed the case for this application where the model correctly predicted that the 40 KDa PEG and PEGylated 50 KDa protein Rh values were not significantly different to enable their separation by SE-UPLC. However, in many instances and for this application SE-UPLC can be used to separate the modified and unmodified protein components of the sample, particularly for samples where large MW PEG (20 and 40 KDa) are being used. By comparison, for this specific application it was found that all the three components were well separated in a single analysis based on differences in their hydrophobicities using a 300Å pore size bridged ethyl hybrid (BEH) column at using a TFA and acetonitrile mobile phase and at a relatively high temperature (90 ˚C).

Stability of BIOshell A400 Protein C4 Columns at Elevated Temperatures

Hillel Brandes
Sigma Aldrich/Supelco
Bellefonte, PA
hillel.brandes@sial.com

Coauthors: Dave Bell, Roy Eksteen

Abstract:
In general, when analyzing compounds of low molecular mass by HPLC, increasing temperature results in higher column efficiency, improved peak shape and shorter analysis time, at the expense of a reduction in selectivity. Recently we reported the initial results of the reversed-phase HPLC analysis of monoclonal antibodies at temperatures up to 90°C [1]. While we found that the peak shape of most proteins improved as expected, we also noted that several commercial columns did not provide adequate peak shape for recombinant monoclonal antibodies at their maximum recommended operating temperature. Among the columns we evaluated, the wide pore columns packed with superficially porous particles (SPPs) provided higher peak heights, narrower peak widths, and in some cases higher peak areas, possibly because of the smaller surface area of superficially porous particles. In this follow up study we investigated the long-term stability of BIOshell™ A400 Protein C4 columns, packed with 3.4 µm, 400Å pore size, Fused-Core™ particles for the analysis of peptides and proteins. The results of our study shows that columns packed with Fused-Core™ particles can be used reliably at very high temperatures when needed for the analysis of biotherapeutic drugs and other proteins. Fused-Core columns not only allow the user to reduce analysis time over columns packed with fully porous particles of the same size, they will do so for thousands of column volumes.

[1] Roy Eksteen, Dave Bell, Hillel Brandes, HPLC 2014, New Orleans, LA, Poster P-W-2314, Using Temperature to Improve Peak Shape of Hydrophobic Proteins in RP-HPLC.

Analyses of chlorinated contaminants in food products by atmospheric-pressure dissociative electron attachment ionization

Carina Minardi
Georgetown University
Arlington, VA
cm686@georgetown.edu

Coauthors: Paolo Lecchi, Kaveh Jorabchi

Abstract:
Chlorinated contaminants in food products may arise during refining processes. An example of such compounds is monochloro-1,2-propanediol (3-MCPD) known to have carcinogenic effects. 3-MCPD is found in such food products as vegetable oils, baby formula, and soy sauces. Here we report atmospheric-pressure dissociative electron attachment (DEA) as an alternative ionization approach to enable LC analyses and simple sample preparation methods for chlorinated food contaminants. An LC-MS method was developed with a C18 Aeris Peptide column at a solvent flow rate of 200 µL/min and separation of 3-MCPD and chloroethanol (internal standard) in 5.3 and 5.9 minutes, respectively. The test matrix for this study was soy sauce, in which a simple liquid-liquid extraction of ethyl acetate was optimized for 3-MCPD extraction. Data have yielded reproducible and linear extraction efficiencies for 3-MCPD and chloroethanol (48.8%) with a limit of detection of ~30ppb. Furthermore, a range of soy sauce matrices have been employed to examine the effect of matrix variation.

Plasma-assisted reaction chemical ionization for high-sensitivity elemental quantification of halogens in organic compounds

Haopeng Wang
Georgetown University
Washington, DC
hw240@georgetown.edu

Coauthors: Ninghang Lin, Kaveh Kahen, Hamid Badiei, Kaveh Jorabchi

Abstract:
Pesticides, fire retardants, and many other widely used halogenated organic compounds have attracted attention due to their neurotoxicity and genotoxic effects to humans and animals. [1] Importantly, these halogenated chemicals are also known for their persistence in the environment and bioaccumulation through food chains. [2] Several analytical methods have been developed for detection and characterization of halogenated organic compounds. In particular, elemental mass spectrometry is appealing because it enables quantitative detection without individual standard for each compound. However, conventional inductively coupled plasma – mass spectrometry (ICP-MS) suffers from low sensitivity in detection of halogens because of their high ionization potentials. [3] We have recently introduced plasma-assisted reaction chemical ionization mass spectrometry (PARCI-MS) for elemental analysis of halogens in organic compounds. [4,5] It has shown great promise for highly sensitive detection of bromine via improvements in ionization and ion sampling. Here, we couple a gas chromatography (GC) to the PARCI-MS. Uniform response factor for bromine across eight tested brominated compounds of drastically different chemical structures confirms PARCI's ability to quantify organobromines in the absence of individual standards. Over 3 orders of magnitude linear dynamic ranges is demonstrated for bromine quantification. A detection limit of 30 fg of bromine on column is achieved using GC-PARCI-MS, ~4-fold improvement over ICP-MS.

References: [1] H. S. Hendriks, M. Meijer, M. Muilwijk, M. van den Berg, R. H. Westerink, Arch. Toxico., 88, 857 (2014); [2] A. K. Venkatesan, R. U. Halden, Sci. Rep., 4, 3731 (2014); [3] R. Houk, Anal. Chem., 58, 97A (1986); [4] H. Wang, N. Lin, K. Kahen, H. Badiei, K. Jorabchi, J. Am. Soc. Mass Spectrom., 25, 692 (2014); [5] N. Lin, H. Wang, K. Kahen, H. Badiei, K. Jorabchi, Anal. Chem., 86, 7954 (2014).

Characterization of proanthocyanidin content in dietary supplement Standard Reference Materials by two-dimensional liquid chromatography with high-resolution mass spectrometry

Benjamin Place
National Institute of Standards & Technology
Gaithersburg, MD
benjamin.place@nist.gov

Coauthors: Catherine Rimmer

Abstract:
Proanthocyanidins are an important, and complex, class of polyphenolic compounds with studied health benefits. Individual identification of these oligomeric compounds in mixtures, such as dietary supplements, can be difficult, yet identification may provide insight to their contribution toward overall human health. Separation and detection of the proanthocyanidins was performed through the hyphenation of two-dimensional liquid chromatography with a hybrid quadrupole-Orbitrap mass spectrometer. The offline two-dimensional separation method included the automated fraction collection of eluent from the reversed-phase separation, followed by the subsequent re-injection of the individual fractions onto a HILIC column for orthogonal separation and introduction into the mass spectrometer. Preliminary computational tools were developed for the handling of the mass spectrometric data, but with the advanced MS experiments processing and display can be cumbersome and requires conversion of MS files to common data formats that are less memory-efficient. The use of NIST Standard Reference Materials (SRMs) of dietary supplements, including green tea, bilberry, and cranberry extracts, were used for the demonstration of the method.

Development of Candidate Standard Reference Material® 3949 Folate Vitamers in Frozen Human Serum

Johanna Camara
National Institute of Standards & Technology
Gaithersburg, MD
johanna.camara@nist.gov

Coauthors: Mark S. Lowenthal, Jeanita S. Pritchett, Karen W. Phinney, Lane C. Sander

Abstract:

The National Institute of Standards and Technology (NIST) provides a variety of Standard Reference Materials (SRMs) for the analysis of nutrient levels in clinical matrices. NIST currently offers SRM 1955 Homocysteine and Folate in Human Serum, with certified values for homocysteine and 5-methyltetrahydrofolate (5-mTHF) and reference values for folic acid, also known as pteroyl-glutamic acid (PGA), over three concentration levels of material. Once out of stock, this SRM will be replaced with a new candidate material, SRM 3949 Folate Vitamers in Frozen Human Serum. This new SRM will have three concentration levels with low, medium, and high certified values for both 5-mTHF and PGA. NIST also intends to assign reference values for the additional minor folate metabolites tetrahydrofolate (THF), 5-formyltetrahydrofolate (5-fTHF), 5,10-methenyltetrahydrofolate (5,10-methenylTHF), and the oxidation product of methyl folinate (MeFox). To produce SRM 3949, pilot sera were collected from 15 individual donors, five of which were given a 400 µg folic acid supplement one hour prior to blood draw in an attempt to increase serum levels of 5-mTHF and PGA for the high level material without the requirement for additional spiking. To stabilize the folates, 0.5 % (w/v) ascorbic acid was added as soon as possible after collection of serum. These pilot sera were screened for five folates plus the oxidation product, MeFox, at the CDC by ID-LC-MS/MS. Screening results ranged from 5 nmol/L-72 nmol/L for 5-mTHF, 0.4 nmol/L-32 nmol/L for PGA, 0.25 nmol/L-2.2 nmol/L for THF, and 0.13 nmol/L-3.2 nmol/L for MeFox. Both 5-fTHF and 5,10-methenylTHF were below the limits of detection for all sera. Four pilot sera from donors administered a folic acid supplement displayed significantly elevated levels of both 5-mTHF and PGA. Based on these results, a blending protocol was specified to obtain the desired folate concentrations in each of the three SRM 3949 concentration levels. The endogenous levels of 5-mTHF and PGA in all three concentration levels and enhanced folate stability via ascorbic acid addition are improvements over the original SRM 1955 that should better serve the end users. The candidate material has been blended and packaged and will undergo additional analyses by ID-LC-MS/MS at both NIST and the CDC.

PEGylated Gold Nanorod Separation and and Characterization by A4F based on Aspect Ratio with UV-Vis Detection

Liz Nguyen
National Institute of Standards & Technology
Gaithersburg, MD
thao.nguyen@nist.gov

Coauthors: Julien Gigault, Vince A. Hackley

Abstract:
Asymmetric-flow field flow fractionation (A4F) is utilized as an elution based separation technique employed for the characterization of gold nanorods (GNR). For the successful continual development of biological applications of GNRs, knowledge of the aspect ratio (AR) distribution of GNRs modified with polyethylene glycol (PEG) and its chain structure is important. GNR of varying AR were obtained both commercially and synthesized using template methods. The nanorods are functionalized with PEG having different molar masses. The development of an A4F separation and characterization methodology for PEGylated GNR in a non-cytotoxic mobile phase is demonstrated. These new A4F results identically follow the results from a previously established methodology by Gigault et al. in which, the parameter that principally controls elution of GNRs is their aspect ratios. The developed methodology applied to biocompatible GNRs can be utilized in the future for purification and characterization of GNR for in vivo AR specific therapy and treatments.

LC-MS/MS: A Tool to Mitigate Interferences in Complex Matrices

Carolyn Burdette
National Institute of Standards & Technology
Gaithersburg, MD
carolyn.burdette@nist.gov

Coauthors: Benjamin Place, Johanna Camara

Abstract:

Biological matrices are analytically challenging, due to sample variability and the potential of unforeseen interferences compound the complexity. Presented here is the use of LC-MS/MS capabilities to overcome and mitigate such interferences. NIST investigated an unknown compound that interfered with the quantitative determination of 3-epi-25-hydroxyvitamin-D3 (3epi25(OH)D3) in human serum samples, when analyzed by the NIST Reference Measurement Procedure (RMP). The unresolved peak eluted after the 3epi25(OH)D3 peak, and in different signal ratios for the different samples. A Thermo Q-Exactive Orbitrap mass spectrometer equipped with electrospray ionization (ESI) was used to determine the exact mass of the interfering compound and relevant fragments. Using the HRMS capabilities, the extracted ion chromatograms (EIC) and mass spectra of were examined. One mass in particular seemed to correlate solely with the interfering compound; m/z 419 [M+H]+. The presence of the unknown was determined to be associated with the type of blood collection bag and so initial investigations of the potential interfering compound focused on three masses of interest, m/z 419.31 (diisononyl phthalate, $C_{26}H_{42}O_4$) m/z 419.35 (interferent) and m/z 401.34 (3epi25(OH)D3). While this did not rule out the possibility of a plasticizer as the interferent, it was easy to observe that the exact mass of that particular phthalate, while similar to the interferent mass, does not elute at the same retention time. The capabilities of HRMS allowed for the differentiation of two very similar compounds; diisononyl phthalate (m/z 419.31) and the interference peak (m/z 419.35). Samples containing the interferent were run again on the AB-Sciex API 5000 LC-MS/MS using the RMP with the addition of transitions at m/z 419 —> m/z 401 and m/z 419 —> m/z 383 to monitor the potential interferent. In addition, efforts were made to chromatographically separate the interferent from the 3epi25(OH)D3. A HALO 5 PFP column was used to separate the interference from the analyte and this method will be investigated for use in future vitamin D metabolite measurements in human serum. Based the results found by HRMS and MS/MS and knowledge regarding the source and processing of the human serum, it was hypothesized that the interfering compound was related to specific collection bags. These bags were then obtained and used for additional studies in order to determine the identity of the interference and mitigate the impact of the interference on quantitation of 3epi25(OH)D3.

Separation of Histones from Human Brain

Kyle Anderson
National Institute of Standards & Technology
Gaithersburg, MD
kwa1912@hotmail.com

Coauthors: Jesse Huang, Illarion Turko

Abstract:
Histone post-translational modifications and variants are used to modulate gene expression on the epigenetic level. This level of control, referred to as the "histone code," has been observed in various diseases, particularly cancer and neurodegeneration. A challenge of investigating neurodegenerative diseases, such as Alzheimer's disease, is achieving adequate separation of protein targets from the complex, lipid-rich biological matrix of brain tissue and further separation of isoforms to reduce complexity for analysis. We demonstrate several methods for separating histones and their variants from human brain tissue, which are applicable for subsequent bottom-up or top-down mass spectrometry analysis. Separations include combinations of liquid chromatography, 2D gel electrophoresis, and chemical derivatization of proteins for improved performance.

Highly Stabilized Phospholipid Bilayers as Novel Stationary Phases for Capillary Liquid Chromatography

Elyssia Gallagher
National Institute of Standards & Technology
Gaithersburg, MD
elyssia.gallagher@nist.gov

Coauthors: Elyssia S. Gallagher, Seid Adem, Christopher A. Baker, Saliya Ratnayaka, Craig A. Aspinwall

Abstract:
Transmembrane proteins (TMPs) are mediators of cellular activity, with alterations in TMP structure and function resulting in various debilitating diseases. Current whole-cell, drug screening assays suffer from irreproducibility due to heterogeneous cell populations, exhibit false signals because of non-specific interactions, and are complicated by the various pathways that can be activated during signal transduction. Affinity chromatography is an alternative, synthetic platform in which a single biomolecular target is immobilized, forming the stationary phase. However, affinity chromatography of TMPs requires suspension of the proteins in lipid bilayers to maintain the native conformations. Traditional lipid -based stationary phases suffer from poor chemical, physical, and temporal stability limiting their widespread use. Here we describe the preparation of a highly stable phospholipid bilayer stationary phase prepared by photochemical or redox initiated polymerization of 1,2-bis[10-(2',4'-hexadieoyloxy)decanoyl]-sn-glycero-2-phosphocholine (bis-SorbPC), a synthetic, polymerizable lipid. Based on flow cytometry and fluorescence microscopy, the polymerized bis-SorbPC bilayers exhibited enhanced stability compared to 1,2-dioleoyl-sn-glycero-3-phosphocholine (unpolymerized) bilayers when exposed to chemical (organic solvents and surfactants) and physical (shear forces) insults over a 30 day period. Additionally, frontal chromatographic analyses of acetylsalicylic acid, benzoic acid, and salicylic acid showed increased retention times on columns prepared with polymerized bis-SorbPC stationary phases compared to bare silica particles ($P<0.0001$), indicating that the lipophilic molecules were retained on the polymerized phospholipid bilayer stationary phases.

Preliminary Analysis of Grumbacher Water Mixable Oil Paints

Robyn Hodgkins
National Gallery of Art
Landover, MD
r-hodgkins@nga.gov

Coauthors: Robyn Hodgkins, Barbara Berrie, Suzanne Lomax, Chris Maines

Abstract:
Since the early 1990s, nine lines of water mixable oil (WMO) artists' paints have been introduced to the market. WMOs are formulated from traditional oils with the addition of water and oil miscible components (e.g. emulsifiers, surfactants, stabilizers, dispersants, thickeners). The additives allow the paint to be thinned and cleaned using water, eliminating the use of hazardous solvents (e.g. turpentine). A variety of issues have been reported for contemporary oils that seem to depend on manufacturer and decade produced (e.g. metal soap efflorescence, magnesium sulfate formation, delamination, liquefaction). Little is known about how the chemical and physical properties of WMO paints compare to these contemporary oils. We have begun investigating Grumbacher Pre-tested oils and Grumbacher Max water mixable oils as a starting point. We are determining the best derivatization agents and analysis methods to identify the additives in both traditional oil and WMOs. We have found that to identify unoxidized additives, polyethoxyethylene sorbitol hexaoleate and castor wax, the sample should be methylated with triammonium hydroxide before analysis by GC-MS. Other derivatization agents (TMTFTH, TMSH) produced side products. These results will be used for assessing the involvement of additives on the curing process compared to traditional paints.

Bio-Monolith Protein A Column for Monitoring of mAb Biosimilar titers: Cell Culture Optimizations and Selection

Nicole Hart
Agilent Technologies
Wilmington, DE
nicole_hart@agilent.com

Coauthors: James Martosella, Phu Duong, Maureen Joseph, Emmie Dumont, Isabel Vandenheede, Pat Sandra, Koen Sandra

Abstract:
In the biopharmaceutical industry, downstream processing for monoclonal antibody productions typically include three chromatographic steps; capture, intermediate purification and polishing. Protein A chromatography is frequently used as a capturing step for monoclonal antibodies (mAb) from cell culture supernatants resulting in excellent throughput (i.e., capacity and speed). Additionally, protein A chromatography plays an important role to concentrate the target molecule- immunoglobulin. To monitor mAb titer and yield from cell culture supernatants, analytical measures are necessary during processing to determine the optimal time for harvesting mAb production before expensive preparative scale separations are employed. In this work, Agilent Bio-Monolith Protein A Columns were used to illustrate the separation and monitoring of mAb titer and yield for cell-clone selection of a Trastuzumab (Herceptin) biosimilar. The protein A purification was followed by mass spectral (MS) analysis to demonstrate the power of the Bio-monolith Protein A column for cell culture selection and optimization for tuning of mAb glycosylation profiles. Additionally, the developed methods show the injection linearity, robustness and speed of the Bio-monolith column.

A Novel HILIC Column for High Speed N-linked Glycan Analysis

Nicole Hart
Agilent Technologies
Wilmington, DE
nicole_hart@agilent.com

Coauthors: James Martosella, Oscar Potter, Danny Mancheno, Jia Liu

Abstract:
Recombinant monoclonal antibody therapeutics (mAbs) represents the largest group of therapeutic proteins. The efficacy of these therapeutics are highly dependent on the correct glycosylation patterns of the mAbs and so far, all licensed therapeutic mAbs are immunoglobulines G (IgGs). Human IgG has a single conserved N-linked glycosylation site located on each heavy chain. N-linked glycosylation is a critically important and an elaborately complex post-translational modification that requires control, monitoring and understanding during all phases of glycoprotein drug development, processing and manufacture. Properties like safety, efficacy and the serum half-life of therapeutic proteins can be affected by differences in their glycosylation pattern. Therefore, analysis of the glycan pattern is an important part of characterization of therapeutic glycoproteins, especially mAbs. Different strategies have been applied for the analysis of glycans. However, the majority of methods are based on enzymatic protein release of glycans from the mAb with subsequent derivatization with a labeling agent, such as 2 aminobenzamide (2-AB). Due to the lack of chromophores on the glycan, labeling with a 2-AB (neutral) tag enables fluorescence detection. Labeled glycans are very hydrophilic structures and the preferred separation technique is by Hydrophilic Interaction Chromatography, commonly referred to as HILIC. Separation using HILIC with fluorescence detection is a robust method for glycan analysis while HILIC-LC can also be coupled to mass spectrometry for obtaining important mass and structure information. In this work, we introduce the AdvanceBio Glycan Mapping Column, a new sub-2um HPLC column with a novel HILIC chemistry for high-throughput glycosylation analysis. The column and methods provide high resolution of glycans with a 60% reduction in elution time compared to currently available HPLC column technologies. To illustrate the utility of AdvanceBio Glycan Mapping, we studied a 2AB labeled human IgG glycan sample. Quantification and glycan structural assignments were obtained using fluorescence detection.

Exploring microLC/MS/MS for Accelerating Peptide Quantitation Assays

George Manning
AB Sciex
South Riding, VA
george.manning@absciex.com

Coauthors: Leo Wang; Christie Hunter; Khaled Mriziq; Remco Van Soest; Subodh Nimkar

Abstract:
In this poster we will show how switching from nanoLC/MS to microLC/MS can improve throughput significantly for sensitive peptide quantitation.

Rapid Quantitation of Substance P in Plasma Using Differential Mobility Spectrometry and Micro-flow Chromatography

George Manning
AB Sciex
South Riding, VA
george.manning@absciex.com

Coauthors: Daniel M. Warren and Sushmit Maitra

Abstract:
Substance P is an 11 amino acid neuropeptide that is known to modulate neural responses primarily associated with pain perception. Recent studies have shown that this peptide also plays a significant role in regulating the immune system, and that its increased production is part of the pathology of several autoimmune/inflammatory disorders including Inflammatory Bowel Disease and Rheumatoid Arthritis. Consequently, there is significant interest in analytical strategies that enable detection of Substance P at physiologically relevant concentrations. Here we describe a fast and robust method to detect and quantitate Substance P in protein precipitated plasma. We demonstrate that sub-femtomole limits of quantitation (LOQs) are obtained by combining traditional Multiple-Reaction Monitoring with micro-flow liquid chromatography and Differential Mobility Spectrometry (DMS).

Separation of Cycloartane Glycosides from the African Plant *Sutherlandia frutescens* using High-Speed Countercurrent Chromatography

Martha Knight
CC Biotech LLC
Rockville, MD
marthaknight@starpower.net

Coauthors: Cuiping Chen, Moon-Jun Brian Kim, Thomas M. Finn, William F. Folk

Abstract:
In a butanol extract of the Sutherlandia frutescens various cycloartane glycosides, termed sutherlandiosides and sutherlandins have been characterized. The plant extracts are used in traditional medicine and are considered to have anti-inflammatory and anti-cancer activities. The methods to purify the 4 known sutherlandiosides are multistep large volume extractions followed by column separations resulting in low yields. We have purified, sutherlandioside B in one step using countercurrent chromatography with the spiral tubing support rotor to obtain high recovery [ ] of this major metabolite. We are now developing solvent systems to fractionate each of the other 3 sutherlandioside metabolites. In HPLC separation studies of a methanol extract of the dried plant material we have separated 4 salient flavonoids as well as the sutherlandiosides. At 260 nm the flavonoids are detected whereas the sutherlandiosides can only be detected at the lower wavelength of 220 nm. The partition coefficients of the compounds in various solvent systems can be determined by HPLC analysis of the upper phase and lower phase and calculating the K by peak heights or areas. In plots of Log K vs. the solvent systems greater differences in the K values relate to more separate elutions. The results will be shown graphically. This is an important application of the new spiral rotor used in high-speed CCC for rapid separation of large mass of material to isolate compounds to study their biologic activity.

WCDG Membership

Are you interested in becoming a member of the WCDG? Membership dues are only $10 per year (Sept-May) and can be paid to the Treasurer, Al Del Grosso, at any meeting.

Dues can also be paid online at any time at http://wcdg.squarespace.com (find the "Pay Now" button on the right menu bar)

WCDG Mailing List

To be added to the WCDG mailing list, visit our website at http://wcdg.squarespace.com (find the "Subscribe" button on the right menu bar).

WCDG Corporate Sponsorship Program

Are you interested in becoming a corporate sponsor of the WCDG? We offer many levels of corporate sponsorship to fit the needs of any chromatography company, big or small. Visit our sponsorship site at http://wcdgsponsorship.ezregister.com for more information about the sponsorship levels and to join our growing corporate sponsorship program.

Questions about sponsorship? Contact board members Jonathan Edelman (jonathan.edelman@gmail.com), Bob Swart (swartrp@hotmail.com), or Nicole Hart (nicole_hart@agilent.com) for more information.

WCDG Programming

Do you have program or speaker ideas? To make suggestions or offer to give a presentation to the WCDG, contact the Program Chair, Ashraf Khan, at ak@usp.org.

Future WCDG Meeting Dates

The WCDG holds regular meetings, generally on the third Wednesday of each month from September through May at the US Pharmacopeia in Rockville, Maryland. (Some variability in day and location) At 6:00 p.m. a light dinner is served followed by the featured speaker at 7:00 p.m. We welcome you to join in our discussions. Visit our website (http://wcdg.squarespace.com) for updates and additional information.

Tentative 2014-2015 Meeting Dates and Locations

Wednesday, October 15, 2014
US Pharmacopeia, Rockville, MD

Wednesday, November 12, 2014
US Pharmacopeia, Rockville, MD

Wednesday, December 17, 2014
US Pharmacopeia, Rockville, MD

Wednesday, January 21, 2015
US Pharmacopeia, Rockville, MD

Wednesday, February 11, 2015
US Pharmacopeia, Rockville, MD

Tuesday, March 10, 2015
The Howlin' Wolf, New Orleans, LA

Wednesday, April 15, 2015
US Pharmacopeia, Rockville, MD

Wednesday, May 20, 2015
US Pharmacopeia, Rockville, MD

2014-2015 WCDG Board

Jonathan Edelman,
President & Immediate Past President
jonathan.edelman@gmail.com

Ashraf Khan, Program Chair
ak@usp.org

Melissa Phillips, Secretary
melissa.phillips@nist.gov

Alfred (Al) Del Grosso, Treasurer
alfred.del-grosso@fda.hhs.gov

Peter Howell, Webmaster/Historian
peterbhowell@gmail.com

Catherine (Kate) Rimmer, Member
catherine.rimmer@nist.gov

Robert (Bob) Swart, Member
swartrp@hotmail.com

Nicole Hart, Member
nicole_hart@agilent.com

Ravi Ravichandran, Member
rr@usp.org

Claire Chisolm, Member
cnc@usp.org

Pierluigi Delmonte, Member
pierluigi.delmonte@fda.hhs.gov

Index of Poster Presenters

Notes

WCDG Corporate Sponsors

Silver Level

Agilent Technologies

Bronze Level

ThermoFisher SCIENTIFIC

GERSTEL

J.G. Finneran Associates, Inc.
Design, Engineering and Manufacturing

Spiral CCC™
CC Biotech LLC

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

phenomenex®
...breaking with tradition™

RESTEK

AB SCIEX

SHIMADZU

Metrohm
USA Inc.