POSTER SESSION
& VENDOR EXHIBITION

September 18, 2019
6pm - 8pm

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

Guiochon Student Award

Best student poster will receive the Guiochon Student Award with support to attend HPLC 2020 in San Diego, CA. Winner and Honorable Mentions will receive award certificates and opportunity to present at a WCDG meeting.

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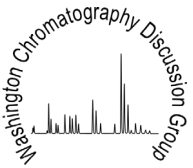

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Table of Contents

Metabolic Characterization of Cell Clones in X. laevis Embryos by HPLC-MS

Jie Li, Peter Nemes
University of Maryland, College Park, MD

Introduction Measurement of metabolic changes as cells differentiate to form specific types of tissues promises to uncover molecular processes underlying tissue induction during early vertebrate embryonic development. Mass spectrometry (MS) provides label-free characterization and quantification of metabolites but current sensitivity limitations require prohibitively large numbers usually thousands to millions of cells for analyses. We here extended metabolomics by MS to identified cell clones that formed in the early developing embryo of Xenopus laevis. The combination of cell lineage tracing whole-embryo dissociation fluorescence-activated cell sorting (FACS) and liquid chromatography (LC) MS enabled us to uncover changes in the metabolic profiles as the midline animal-dorsal cell gave rise to precursor of the neural tissues in this important model in cell and developmental biology. Methods Embryos were obtained by gonadotropin-induced natural mating of adult frogs (IACUC No. R DEC-17-57) dejellied using cysteine and cultured to the 16-cell stage following established protocols. The neural-tissue fated midline animal-dorsal cell (called D11) was identified based on cell morphology and location in the embryo followed by fluorescent labeling of the descendent clone prior to whole-embryo dissociation for FACS. Metabolites were extracted using organic solvents and reconstituted for hydrophilic interaction LC (HILIC) at 130 µL/min and detection using a time-of-flight mass spectrometer (Bruker Qq-TOF). Metabolite identifications were based on accurate mass MS/MS fragmentation and separation time against information available in metabolite databases (Metlin) or measured standards in house. Relative quantification was performed based on under-the-curve peak areas in selected-ion chromatograms. Preliminary Data The neural-tissue fated cell clone was traced in the developing embryo by fluorescently labeling its precursor the D11 cell. These embryos were cultured to the neurula stage and dissociated into a suspension of single cells which measured ~10 to 35 µm in diameter. The labeled cells were sorted by FACS to 95% purity with cell viability confirmed by fluorescence microscopy. Metabolites were extracted from the sorted cells in an aqueous mixture

of methanol and acetonitrile facilitated by periodic sonication and centrifugation to remove cell debris followed by reconstitution of the supernatant in 95% acetonitrile in water. The metabolite extracts were analyzed by HILIC LC-MS optimized for a standard mixture of 13 amino acids (peak tailing factor <1.1). With a demonstrated reproducibility of <1% relative standard deviation (RSD) in separation time and <16% RSD in peak areas this technology was sufficiently robust to detect metabolic changes in developing cell clones in the embryo. XCMS Online (ver. 3.7.1) processing of the MS data revealed 837 molecular features between m/z 50-550, including 30 metabolites that were m/z-matched to known small molecules. Under-the-curve peak areas using extracted ion chromatograms allowed us to compare the metabolic profile of the clone as it developed in the early X. laevis embryo. These results raise a possibility to improve our understanding of metabolism during tissue formation and organogenesis during early vertebrate embryonic development. Text

Titl Online IEX-MS Characterization and Monitoring of mAb Charge Heterogeneity Using an Optimized Cation Exchange Resin and Compact TOF Mass Spectrometer

Annie Landicho, Samantha Ippoliti, Qi Wang, Ying Qing Yu, Matthew A. Lauber
Waters Corporation, Milford, MA

Ion exchange chromatography (IEX) is a method of choice for the analysis of charge heterogeneity encountered with biotherapeutics drug candidates. Traditionally IEX separations require high concentrations of salts that are not compatible with mass spectrometry (MS) analysis which has left a gap in the characterization of charge variants. It has been shown that direct MS-based characterization of these charge variants is possible if volatile salts are employed. In this study MS-compatible ion exchange separations are combined with a new small footprint benchtop TOF MS instrument and applied to a case study on identifying the charge variants formed upon forced degradation.

An Investigation into the Use of Cyclic Ion Mobility for the Separation of Biopharmaceutical Peptide and Protein Modifications

Kevin Murphy, Jim Langridge, Henry Shion, Martin Palmer, Weibin Chen, Dale A Cooper-Shepherd
Waters Corporation, Milford, MA

As biotherapeutics become more complex and companies strive for increased intellectual property protection ever more sophisticated tools are being investigated to provide in-depth detailed molecular characterization. Such studies focus on acquiring knowledge of the post-translational modifications (PTMs) including glycosylation oxidation and deamidation present in the protein product with control of these being paramount. Mass spectrometry (MS) is a central technique in biopharmaceutical characterization due to its ability to report on such a wide range of attributes. However, the presence of isobaric PTMs with differing biological properties can often be refractory to traditional LC-MS workflows even when chromatographically separated. In this work we investigate cyclic ion mobility technology as a means to distinguish isomeric PTMs to improve biotherapeutic characterization.

Discovery of Potential Cancer Biomarkers in Human Plasma Using Comprehensive HRMS

Brad Barrett,[1] David E. Alonso,[1] Habtom Ressom,[2] Cristina Di Poto,[2] Joseph E. Binkley[1]
[1]LECO Corporation, St. Joseph, MI; [2]Georgetown University Medical School, Washington DC

Hepatocellular carcinoma (HCC) is the leading cause of cancer-related deaths worldwide (9th in the USA). Risk Factors: Hepatitis (B & C) alcoholism diabetes obesity and nonalcoholic fatty liver disease. There exists a critical need for early stage intervention and effective medical treatment for HCC. This experiment looks to provide a complement a much larger study investigating metabolite levels in HCC versus liver cirrhosis (CIRR) patients. The ability to identify potential HCC biomarkers using a non-targeted multi-platform approach is examined in this effort.

Development of A SPE LC-MS/MS Method for the Bioanalytical Quantification of Pramlintide from Serum

Amy Hu, Caitlin M. Dunning, Mary E. Lame, Mark D. Wrona, Kim Haynes, Ian Edwards
Waters Corporation, Milford, MA

Pramlintide acetate (SYMLINTM) is a synthetic analogue of the human hormone amylin developed as an adjunctive therapy for patients with type 1 and 2 diabetes. With nearing patent expiry dates for pramlintide and recent research indicating a role for amylin in Alzheimer's Disease models interest in amylin and amylin agonists is rising. Characterized by fast absorption (<30 minutes) elimination (~1 hour) and low circulating levels (pg/mL) amylin agonists such as pramlintide can be challenging to quantify. To date there are no published quantitative assays for this peptide LBA or LC-MS. LC-MS/MS assays have become increasingly popular for peptide quantification due to the high sensitivity and specificity afforded by selective MRM fragments. However, method development and accurate quantification for hydrophobic peptides like pramlintide can still be challenging because they notoriously suffer from non-specific binding (adsorption). This can lead to poor recovery loss of analyte and poor limits of detection. The work described here describes the optimization and development of a selective sample preparation strategy combined with the use of LC-MS compatible sample storage plates with optimized surfaces to mitigate pramlintide loss due to non-specific binding. With this assay an LLOQ of 25 pg/mL of pramlintide was achieved extracted from 100 ÂµL of rat and human serum.

Investigative Multi-Step and Quantitative Analysis of Cannabidiol Oil using the Pyroprobe

Ben Landas, Karen Sam
CDS Analytical, Berryville, VA

With the promise of cannabidiol (CBD) for treating a variety of ailments CBD oil derived from the industrial hemp plant has a growing interest in nutraceutical and pharmaceutical industries. This extract is being credited with helping treat many medical issues. Diluted with hemp oil prior to use CBD oil is a complex natural product containing many volatile and non-volatile constituents. Analytical testing using the Pyroprobe can clarify ingredients in natural materials such as CBD oil by separating ingredients based on their volatility then pyrolyzing the non-volatile portion like the oil itself.

Optimizing MS/MS Acquisition to Generate a Comprehensive Multi-attribute Method Data Archive of the NISTmAb

Michael E. Pettit, John E. Schiel
National Institute of Standards and Technology, Rockville, MD

The multi-attribute method (MAM) allows simultaneous monitoring of a wide variety of biotherapeutic product quality attributes (PQAs). Traditionally, the MAM begins with extensive product characterization via LC-MS based bottom-up tandem MS (MS/MS). The resulting data archive of identified peptides and post-translational modifications (PTMs) are compiled and used in MAM via MS-only workflows (i.e. without MS/MS) to monitor pre-determined PQAs. It is therefore desirable to develop an LC-MS strategy for generating comprehensive data archives for use in MAM evaluation of biotherapeutic products. Here we present a unique MS/MS optimization strategy for expanding the MAM data archive depth using the NISTmAb as a test metric.

Determination of Phenolic Acids in Black Cohosh Standard Reference Materials by Reverse Phase Liquid Chromatography and Photodiode Array Detection

Walter Wilson, Catherine A. Rimmer
National Institute of Standards and Technology, Gaithersburg, MD

Black cohosh (Cimicifuga racemose (L.) Nutt.) roots and rhizomes are used worldwide for the treatment of women's disorders and have been classified as one of the top 10 best-selling herbs in the US. Adulterated black cohosh supplements have been identified and shown to have hepatotoxicity associated with the supplements prepared with other plant parts then the roots rhizomes or different Cimicifuga species. Phenolic acids work as antioxidants to prevent cellular damage due to free-radical oxidation reactions and help promote anti-inflammatory conditions in the human body. In this study a new reversed-phase liquid chromatography (RPLC) method coupled to a photodiode array detector (PDA) was developed for the determination of phenolic acids in four candidate black cohosh Standard Reference Materials. A typical two-dimensional chromatogram based on retention time (x-axis) and absorption (y-axis) is extended to a three-dimensional format with the collection of UV spectra (wavelengths z-axis). The RPLC-PDA method used an ACE 3 C18 column to determine caffeic acid ferulic acid and isoferulic acid in the black cohosh samples. Twelve additional phenolic acid compounds were investigated. Multiple phenolic acids were tentatively identified solely based on retention times. However, the comparison of the UV spectra obtained for reference standards and suspect chromatographic peaks did not match preventing a false identification.

Strategies for the Transfer of Liquid Chromatographic Methods between Different Instruments

M. Grübner, C. Paul, M. De Pra, F. Steiner
Thermo Fisher Scientific, Germering, Germany

A challenging task that frequently occurs in all kinds of analytical industries is the transfer of liquid chromatographic (LC) methods from one instrument to another. This is straightforward in case the transfer is between identical instruments. However, the situation becomes more complicated when instruments of different configurations, generations and/or vendors are used. As all LC hardware components to some extent have influence on the chromatographic results, instrumental differences will also affect the analytical outcome of a transferred LC method. Method robustness as well as the degree of instrumentational deviation determine the analytical deviation. Means to counteract these effects depend on the requirements of the operator. For example, if adequate resolution and congruent quantitative results are obtained and sufficing, no effort in adaption is needed. However, if in addition retention times need to fit exact specifications, the effort might increase. In our study we investigated several strategies to overcome difficulties in method transfer caused by hardware differences between several instrumental platforms. Here our focus was on Thermo Scientific Ultimate 3000 UHPLC Systems, Thermo Scientific Vanquish UHPLC Systems, Agilent Infinity 1260 and Waters Acquity Classic. We examined strategies to modify system dwell volumes such as different mobile phase mixers and sample loop sizes as well as the adjustable delay volume of the Vanquish autosampler. Quaternary and binary systems were considered. The effect of pre-column volumes on peak shape and retention was monitored for strong solvent injections and the impact of detector settings like response time and bandwidth was shown. Means to manage differences in column/eluent thermostatting were illustrated. Recommendations and guidelines for a best practice method transfer are provided.

Charged Aerosol Detection and Method Transfer of Compendial, including USP, Methods

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Thermo Fisher Scientific, Dreieich, Germany; Thermo Fisher Scientific, Germering, Germany; Thermo Fisher Scientific, Chelmsford, USA; Thermo Fisher Scientific, Chelmsford, USA

Detectors most commonly used with HPLC systems include UV detectors and mass spectrometers. HPLC-based regulatory methods frequently rely on the UV detector because it is inexpensive and accurate. The charged aerosol detector is also increasingly being incorporated into regulatory methods because it is inexpensive, easy to use and offers universal detection and uniform response for all non-volatile analytes. This work shows two published United States Pharmacopoeia (USP) methods for charged aerosol detection (CAD) and describes strategies for method transfer between CAD instruments. As part of the USP monograph modernization effort, USP 41(3) In-Process Revision includes a proposed change to the official USP Metoprolol Succinate monograph (USP 38, page 4370) for the determination of organic impurities that lack UV chromophores. The older TLC method is replaced by one that uses a hydrophilic interaction chromatography (HILIC) method coupled with a charged aerosol detector (CAD). This work replicates the updated USP method and related publication, both of which used older models of CAD, and provides guidance for transfer of the method to the new generation CAD. The USP monograph USP 40-NF 35 describes the use of an HPLC-CAD method for the measurement of both deoxycholic acid, its primary impurity, cholic acid, and several minor impurities. This application note replicates the original USP method, which used a Corona ultra RS CAD, and provides guidance for transfer of the method to the new generation Vanquish Flex CAD (VCAD), which is identical to the Corona Veo CAD.

UHPLC-UV Coupled to a Single Quadrupole Mass Detector for Confirmation and Quantification of a Genotoxic Impurity in a Drug Sample

G. Greco, S. Fabel, S. Grosse, M. De Pra, F. Steiner
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During the development phase of new drugs, the determination of process- and product-related impurities is an essential step throughout the lifecycle of a drug. High performance liquid chromatography (HPLC) with ultraviolet light (UV) detection is the most common technique used to assess the purity of an active pharmaceutical ingredient (API). Identification is typically assessed based on retention time. Therefore, single standards have to be run separately. During the early drug development stages, standards of potential impurities are often not available, and the identity of peaks cannot be easily assigned if needed. A special group of impurities are genotoxic impurities, which pose a greater risk to patient health, since they can react with DNA and increase the risk of cancer. The genotoxic impurities identified as potential contaminant of the drug must be monitored and accurately quantified according to rules which are stricter than for other impurities. The United States Food and Drug Administration (USFDA) as well as the European Medicines Agency (EMEA) have established a threshold of toxicological concern (TTC) of 1.5 µg/day for long-term treatments with the drug product [1]. Additionally, the international conference on harmonization (ICH) M7 suggested a staged TTC based on the duration of drug exposure. The developed method demonstrates the ability to monitor and quantify a genotoxic impurity (methyl-p-toluenesulfonate) along with other potentially expected impurities in a drug substance (aprepitant) by UHPLC-UV coupled to a single quadrupole mass detector. Mass-based impurity monitoring has the great advantage of not requiring additional reference standards. Following, accurate quantification of the genotoxic impurity, methyl-p-toluenesulfonate, was done by UHPLC-UV.

Green Synthesis of Gold Nanoparticle-Modified Carbon Fiber Microelectrodes for Enhanced Neurotransmitter Detection

Sanuja Mohanaraj, Pauline Wonnenberg, Alexander G. Zestos
American University, Washington, DC

Gold nanoparticles have received an increased focus due to their biomedical and material applications such as the detection of dopamine. Dopamine is a crucial chemical messenger that plays a pivotal role in the control of movement learning memory cognition and emotion within the nervous system of the human body. The deficiency or surplus of dopamine can cause numerous neurological and psychological interference; among these are Parkinson's Disease (PD) schizophrenia and amphetamine fentanyl and cocaine addiction. However, gold nanoparticles produced from naturally occurring reducing sources have been understudied. In this study the electrochemical detection of neurotransmitters is enhanced by electrodepositing gold nanoparticles (AuNPs) synthesized from the sugars in organic honey onto the carbon fiber (CFME) surface. The sugars (glucose fructose and sucrose) in the raw honey were separated and identified using high-performance liquid chromatography and fructose in particular is hypothesized to be responsible for the reduction of Au^{3+} to Au^{0}. The AuNP-CFMEs improved sensitivity of dopamine detection with respect to unmodified electrodes. The thin and uniform layer of AuNPs on the fiber-surface increased electroactive surface area for dopamine adsorption which lead to considerably higher peak oxidative currents and lower limits of detection. The potential separation of the oxidation and reduction peaks of the AuNP-CFMEs was smaller suggesting higher conductivity and faster electron transfer kinetics which is ideal for measuring the fast-phasic firing of dopaminergic neurons and neurochemical fluctuations in the brain.

Determination of Pesticides in Dog Collars by On-line Supercritical Fluid Extraction – Supercritical Fluid Chromatography - Mass Spectrometry

William Hedgepeth; Yuka Fujito
Shimadzu Scientific Instruments, Inc, Columbia, MD

There has been growing concern about flea collar pesticide effects on animal and human health. Tetrachlorvinphos (TCVP) is an organophosphate insecticide that works by affecting the central nervous system. TCVP is an EPA listed possible carcinogen still used in dog collars. Other collars in the US use a combination of pesticides, such as flumethrin and imidacloprid. On-line supercritical fluid extraction – supercritical chromatography-mass spectrometry (SFE-SFC-MS) is a new analytical technique for the extraction, separation, and detection of compounds in a single analysis that limits the need for extensive manual sample preparation. Extracted compounds are trapped directly onto an analytical column for chromatographic analysis. This technique was applied to the analysis of the above pesticides in store bought dog collars. Extraction of pesticides from dog collars was performed using supercritical CO_2. Extract from the TCVP containing collar was trapped on a 250 mm x 4.6 mm, 5 µm Naphthyl column for SFC analysis. Extracts were performed over a number of days to obtain pesticide release profile of TCVP from the dog collar. Extract from the flumethrin/imidacloprid containing dog collar was sent to a Chiralpak IA-3 column to monitor the isomeric forms of flumethrin. Detection of these compounds was obtained on a triple quadrupole mass spectrometer in positive mode by MRM. A make-up flow containing 10 mM ammonium formate in methanol was used to generate the ammonium adducts of flumethrin that were monitored. Each pesticide could be successfully extracted and analyzed from a cut piece of the dog collar that was placed directly into an extraction vessel without any additional sample preparation. The highest TCVP concentration was obtained two hours after activation of the dog collar. This level gradually decreased over a 48-hour time period until extraction levels remained relatively constant. Constant levels obtained were at 5% of the maximum value found at the two-hour time period. Detection was performed by UV due to the high concentration of TCVP (14.55%) in the dog collar. A flumethrin PESTANAL standard was purchased from Sigma. Four major isomers were observed for standard and flumethrin extracted from the dog collar.

Size heterogeneity of NISTmAb RM 8671 by SEC

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The NISTmAb RM 8671 IgG1κ is intended to provide a well characterized, longitudinally available test material that is expected to greatly facilitate development of originator and follow-on biologics for the foreseeable future. Aggregation is a critical metric to establishing monoclonal antibody consistency and quality due to potential immunogenicity concerns. Therefore, a monomeric purity assay was optimized to evaluate and quantify the presence of aggregates using size exclusion chromatography (SEC). A central composite design optimization was conducted, resulting in a highly robust SEC assay. The optimized SEC method was used to (I) evaluate the homogeneity and stability of RM 8671; (II) assign monomeric purity reference values, and (III) establish the appropriate storage and handling conditions for the material.

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WCDG holds regular meetings, typically on the third Wednesday of each month, from September to May at USP in Rockville, MD (unless otherwise noted). A dinner and social hour begin at 6PM, followed by a speaker at 7PM. We welcome you to join our discussions!

October 16	Paulina Piotrowski (NIST) at USP
November 14	Lane Sander (NIST) at ACS HQ with Chemical Society of Washington
December 18	at USP
January 15	at USP
February 19	at USP
March 18	at USP
April TBA	at Shimadzu Training Center with Washington-Baltimore Mass Spectrometry Discussion Group
May 20	at USP

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