2017

Global CESI-MS Symposium

Boston, MA | October 5-6, 2017

Barnett Institute, Northeastern University

Global CESI-MS Symposium

Barnett Institute, Northeastern University
Boston, Massachusetts, USA | October 5-6, 2017

Host:

Professor Alexander R. Ivanov
Barnett Institute of Chemical and Biological Analysis, Northeastern University, USA

Session Chairs:

Professor Alexander R. Ivanov
Barnett Institute of Chemical and Biological Analysis, Northeastern University, USA

Professor Jennifer Van Eyk
Cedars-Sinai Medical Center, USA

Professor John R. Yates III
The Scripps Research Institute, USA

Dr. Spencer Walse
USDA-ARS, USA

Sponsored by SCIEX

Day 1 – Proteomics & Metabolomics

Time	Description
08:00 – 09:00	**Registration**
09:00 – 09:15	**Welcome and Introduction** *Symposium Chair and Your Host, Professor Alexander R. Ivanov, Barnett Institute of Chemical & Biological Analysis, Department of Chemistry & Chemical Biology, Northeastern University*
09:15 – 09:30	**Opening Remarks** *Kenneth W. Henderson, Professor & Dean, College of Science, Northeastern University, USA* *Michael Pollastri, Professor & Chair, Department of Chemistry & Chemical Biology, Northeastern University, USA*
Proteomics & Metabolomics | *Session Co-Chairs: Professor Alexander R. Ivanof and Professor Jennifer Van Eyk*	
09:30 – 10:10	**Profiling B-Type Natriuretic Peptide Cleavage Peptidoforms in Human Plasma by CESI-MS** *Professor Jennifer Van Eyk, Cedars Sinai Medical Center, USA*
10:10 – 10:50	**N-Glycan Analysis in Positive Ionization Mode with CESI-MS** *Guinevere Kammeijer, MSc, Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands*
10:50 – 11:10	**Break**
11:10 – 11:50	**A Sensitive and Versatile Metabolomics Methodology by CESI-MS** *Dr. Bryan Fonslow, SCIEX, USA*
11:50 – 12:30	**Improving Detection Limits in Peptide Analysis with CESI-MS** *Dr. Steve Lock, SCIEX, UK*
12:30 – 13:30	**Lunch**
13:30 – 14:10	**Single-Cell DNA Methylation Profiling Using CESI-MS/MS** *Professor Xinxiang Zhang, Peking University, China*
14:10 – 14:50	**Characterization of Exchangeable Apolipoproteins by CESI-MS from Size Fractionated Human Plasma** *Dr. David Schieltz, Centers for Disease Control, USA*
14:50 – 15:30	**Break – Posters**
15:30 – 16:10	**CESI-MS Tips and Tricks** *Dr. Bryan Fonslow, SCIEX, USA*
16:10 – 17:00	**Lab Tour** – *Sign-up during breaks*
18:00 – 20:00	**Union Oyster House Reception** *Join us for a cocktail reception at the historic Union Oyster House, 41 Union St, Boston, MA 02108*

09:30 – 10:10

Professor Jennifer Van Eyk
Cedars Sinai Medical Center, USA

Profiling B-Type Natriuretic Peptide Cleavage Peptidoforms in Human Plasma by CESI-MS.

INTRODUCTION: B-type Natriuretic Peptide (BNP) is a biologically active circulating hormone. The BNP plasma concentration is routinely used in the diagnosis of heart failure. Antibody-based methods are traditionally used for clinical analyses of BNP, but these cannot distinguish proteolytic variants of BNP. Capillary electrophoresis-electrospray ionization-MS (CESI-MS) can produce high-resolution separations based on the uniqueness of molecular electrophoretic mobility. Neutral coated capillaries are well suited to proteolysis analysis and can handle plasma-derived clinical samples. Finally, CESI-MS can accommodate multiple successive sample injections simultaneously analyzed in one run. Our purpose is to efficiently apply CESI-MS to analytically define multiple BNP proteolytic variants, with a view to develop improved diagnostic parameters for heart failure.

METHODS: Artificial plasma is made of PBS containing 1 mmol/L AEBSF and supplemented with 45 g/L BSA. Both artificial and human plasma were centrifuged for lipid removal and filtered by 0.45um spin column. Recombinant BNP1-32 (B5900) from Sigma Aldrich was spiked in artificial and human plasma (Bioreclamation Inc). The samples were separated on a neutral coated CESI capillary with the CESI 8000 Plus High Performance Separation-ESI platform (SCIEX), which was coupled to and analyzed with a QEplus MS instrument (Thermo) in MS1 scanning mode with a resolution of 70000.

PRELIMINARY DATA: We developed a novel approach in which BNP cleavage peptidoforms are profiled using CE-MS as they are generated over time in minimally processed plasma. In our approach, standard BNP is spiked into a plasma sample, where endogenous peptidases cleave BNP into its peptidoforms. This reaction can proceed in a CE sample vial, and sampled after any desired time interval. When combined with multi-segment injection (MSI), this method allows for the parallel analysis of multiple plasma samples, with successive CESI-MS runs providing a time course for BNP proteolysis profiling. Similarly, MSI can be used to produce a multi-point BNP peptidoform profile from a single plasma sample across the protracted timeframe of a single CESI-MS run.

NOVEL ASPECT: Our CESI-MS approach can simultaneously inject multiple samples to detect proteolytic variants of BNP in a single capillary run.

10:10 – 10:50

Guinevere Kammeijer, MSc,
Leiden University Medical Center, The Netherlands

Guinevere S. M. Kammeijer, Noortje de Haan, Sander Wagt, Pablo Mohaupt, Manfred Wuhrer
Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands

N-Glycan Analysis in Positive Ionization Mode with CESI-MS

INTRODUCTION: Glycosylation analysis is relevant for both the biopharmaceutical industry as well for clinical biomarker discovery. Various properties and activities of proteins are influenced by glycosylation. Even though there are many different methods developed for studying the carbohydrate modifications of a protein, there are still some challenging tasks left. One of these is the identification of linkage isomers, which is often hard to obtain by tandem mass spectrometry alone. Capillary electrophoresis coupled to mass spectrometry via electrospray ionization with a sheathless interface (CESI-MS) is an attractive platform that could be used to tackle this issue. Furthermore, in combination with a dopant enriched nitrogen gas this technique is highly sensitive.

METHODS: For this study N-glycans were released from total plasma with PNGase F. The CE separation selectivity was improved by neutralizing the negative charge of the sialylated glycans with a derivatization step. A cationic charge was implemented on the N-glycans by introducing a label on the reducing end. CE-ESI-MS experiments were carried out in positive ion mode with an in-house neutrally coated capillary or commercially available neutrally coated capillary. By investigating a "zero-flow" principle (i.e., without applied pressure but with applied capillary voltage) the method was further developed with a special focus on the separation of galactose positional isomers (e.g. alpha3- versus alpha6-arm galactosylation).

PRELIMINARY DATA: Two different capillaries were investigated for their use in analyzing positively labeled N-glycans. Both capillaries revealed a two fold increase in signal-to-noise ratio when a dopant enriched nitrogen gas was implemented. The neutral capillary with static coating (commercially available) showed an overall better separation than with dynamic coating (in-house coated). This appears to be mainly due to the fact that the electro osmotic flow (EOF) is more efficiently suppressed with the static coating. Notably, due to the absence of EOF, a small pressure (flow) had to be applied to create a stable electrospray which affected the separation in a negative way.

During this study we further investigated the possibility to create the flow (applying pressure) as late as possible to enhance the separation, the so called "zero-flow" principle, by temporarily switching off the pressure as well the electrospray ionization. Preliminary results revealed the potential of using the zero-flow principle with static coating to separate galactose positional isomers. Additionally, this study shows that the analysis of released N-glycans with CESI-MS is possible in positive ion mode after derivatizing the sialic acids and addition of a positive reducing-end label. Further research will determine the limit of detection and compare the sensitivity with already available negative ion mode CE-MS platforms.

11:10 – 11:50

Dr. Bryan Fonslow
SCIEX, USA

A Sensitive and Versatile Metabolomics Methodology by CESI-MS

INTRODUCTION: Metabolomics represents the analysis of a wide class of primarily small molecules important in biology. Analysis of a subset of metabolites, those that are charged and polar, are particularly challenging since they are typically not universally retained or resolved on traditional LC columns. Additionally, many have structural isomers that can't be resolved as isobars by mass spectrometry (MS) alone. Capillary electrophoresis presents the capabilities to separate many charged and polar metabolites with a single separation condition. CESI is the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device, offering several advantages including increased ionization efficiency and a reduction in ion suppression at low nL/min flow rates. We describe CESI-MS methodologies in both positive and negative ESI mode for the sensitive analysis of charge and polar metabolites involved in central carbon metabolism and neurotransmission.

METHODS: A method was established for detection of over 50 metabolites using positive and negative ion CESI-MS. This work used normal and reverse polarity with a pressure injection and a background electrolyte of 10% acetic acid. Electrophoretic separation was performed using 30 kV creating a field strength of 333V/cm. CESI was coupled to a SCIEX TripleTOF® 6600 and QTRAP® 6500 mass spectrometers operating in positive and negative ion electrospray mode using full scans and looped dedicated product ion scan for each of the metabolites together with a general TOF scan to screen for unknown targets. The total scan time for each cycle was approximately 1 second. The ion spray voltage was set to 1 or - 1 kV.

PRELIMINARY DATA: Both normal and reverse polarities were investigated. Preliminary trials showed that normal and reverse polarity provided the best separation on the CESI-MS system in positive and negative ESI modes, respectively. Under these conditions a lower ion spray voltage could be used which reduced the potential for discharge formation in negative ESI mode.

Using an OptiMS bare fused silica cartridge and 10% acetic acid as a background electrolyte, low nM concentration of metabolites were detected. These metabolites included low molecular weight organic acids including succinate and malate. In addition, separation of the isobaric phosphorylated disaccharides of sucrose-6-phosphate and trehalose-6-phosphate was possible and the partial separation of phosphorylated monosaccharides were observed in the same separation.

This study investigated both separation reproducibility and sensitivity for numerous metabolites difficult to analyze by other means.

11:50 – 12:30

Dr. Steve Lock
SCIEX, UK

Stephen Lock,[1] Jim Thorn,[1] Bryan Fonslow[2] and Edna Betgovargez[2]
1 SCIEX, UK
2 SCIEX, USA

Improving Detection Limits in Peptide Analysis with CESI-MS

INTRODUCTION: There are several important basic neuropeptides which include Vasoactive intestinal peptide (VIP), Pituitary adenylate cyclase-activating polypeptide (PACAP), and Parathyroid Hormone (PTH). These peptides are generally very basic (with isoelectric points > 10) and are difficult to analyze by LC-MS methods as they bind to columns and auto-samplers and have very poor chromatographic properties. CESI is the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device. CESI-MS operates at low nL/min flow rates offering several advantages including increased ionization efficiency and a reduction in ion suppression. In this work we describe the use of the electrokinetic injection technique to improve sensitivity in a CESI-MS method for the analysis of these intact peptides.

METHODS: Peptides standards were prepared in a variety of different solvents and injected by either pressure or electrokinetically onto a 30 µm ID, 91 cm long coated capillary with positive or neutral surface. To check the applicability of this approach to biological matrices, serum was extracted using a common solid phase extraction protocol previously developed. The CE separation used normal or reverse polarity with a background electrolyte (BGE) consisting of a mixture of Acetic acid, Acetonitrile and Water. The MS system was fitted with the NanoSpray® III source and was used in MRM mode at an ionspray voltage of 1600 - 1800 V to detect the peptides.

PRELIMINARY DATA: A CESI-MS method has been developed with the capability of detecting neuropeptide standards at biologically relevant levels (<1 pg/µL). An initial study showed that a transient isotachophoresis (tITP) injection technique was capable of detecting these peptides at <1 pg/µL but when compared with electrokinetic injection (EK), the EK approach provided an additional 5-10 fold improvement in sensitivity. This improvement was dependent on the level of charge of the peptide and the percentage of organic solvent in the sample.

In a previous study,[1] we had used a bare fused silica capillary, covalently coated with Polyethylenimine (PEI) to prevent sample adhering to the column. This positively charged capillary had injection to injection times > 25 minutes. The new method was developed using a neutrally coated capillary of the same dimensions. Due to the change in coating and modifications to the method runtimes, the new method runtimes have now been reduced to < 20 minutes.

NOVEL ASPECT: Electrokinetic injection technique used to enhance CESI-MS detection of basic neuropeptides.

1. Lock, S. 'The application of Capillary Electrospray Ionization to the detection of Neuropeptides'. Conference Proceedings ASMS 2016, San Antonio, June 5-9, 2016.

13:30 – 14:10

Professor Xinxiang Zhang
Peking University, China

Fang YUAN,[1] Xiaohui ZHANG,[1] Hongxu CHEN,[2] Yinglin ZHOU,[1] Xinxiang ZHANG[1]
1 Beijing National Laboratory for Molecular Sciences (BNLMS), MOE Key Laboratory of Bioorganic Chemistry and Molecular Engineering, College of Chemistry, Peking University, Beijing 100871, China
2 Shanghai AB Sciex Analytical Instrument Trading Co., Ltd, Beijing. P. R. China

Single-Cell DNA Methylation Profiling Using CESI-MS/MS

INTRODUCTION: DNA methylation and demethylation are important epigenetics phenomena which have been proved to be connected with many fundamental physiological processes in recent years.[1-2] A number of analytical methods have been developed to sensitively determine the absolute levels of 5-mdC, 5-hmdC, 5-fdC and 5-cadC in different kinds of biological samples.[3-6] As precision medicine and liquid biopsy rise, more and more scientists become interested in the modification of nucleosides in cfDNA and DNA in CTC (circulating tumor cell). It is a great challenge to analysis DNA modifications in such small amount of samples. In this study, a single-cell DNA 5-mdC and 5-hmdC quantification method was developed for the first time, by using the novel CESI-MS/MS technique.

METHODS: CESI-MS/MS sample collection by capillary trap system.

PRELIMINARY DATA: The comparison of the LODs of four DNA modifications using HPLC-MS and our CESI-MS/MS method.

LODs	5-mdC	5-hmdC	5-fdC	5-cadC
HPLC-MS (fmol)	1.25	2.50	0.40	0.40
labelling (fmol)	0.10	0.06	0.11	0.23
This tech (amol)	0.40	0.80	0.08	0.08

1 A. Bird, *Genes Dev,* 16. 6-21 (2002).
2 M. R. Branco, G. Ficz and W. Reik, *Nat Rev Genet,* 13. 7-13, (2012).
3 L. Zhang, L. Zhang, K. Zhou, X. Ye, J. Zhang, A. Xie, L. Chen, J. X. Kang and C. Cai, *J Biomol Screen,* 17. 877-884, (2012).
4 M. L. Chen, F. Shen, W. Huang, J. H. Qi, Y. Wang, Y. Q. Feng, S. M. Liu and B. F. Yuan, *Clin Chem,* 59. 824-832, (2013).
5 R. Yin, J. Mo, M. Lu and H. Wang, *Anal. Chem.,* 87. 1846-1852, (2015).
6 Y. Tang, S. J. Zheng, C. B. Qi, Y. Q. Feng and B. F. Yuan, *Anal Chem,* 87. 3445-3452, (2015).

14:10 – 14:50

Dr. David Schieltz
Centers for Disease Control and Prevention, USA

David Schieltz, Bryan A. Parks, Yulanda Williamson, Kevin Bierbaum, John Barr, Zsuzsanna Kuklenyik,
Centers for Disease Control and Prevention, ~~Atlanta, GA~~

Characterization of Exchangeable Apolipoproteins by CESI-MS from Size Fractionated Human Plasma

INTRODUCTION: Lipoproteins from human plasma were separated by size using Asymmetric Flow Field Flow Fractionation (AF4) to yield 40 fractions that contained HDL, LDL and VLDL particles in a 7-36 nm size range with a resolution of ~1 nm. Aliquots from each fraction were processed and analyzed using LC-MS/MS to quantify apolipoproteins, non-polar and polar lipids. Analyte concentration versus size profiles were constructed. With this approach, individuals with various dyslipidemia were characterized and compared with normolipidemic individuals. To complement the quantitative data, intact proteins in each of the HDL fractions were analyzed by capillary zone electrophoresis and tandem mass spectrometry to characterize the intact exchangeable apolipoproteins.

METHODS: Parameters for background electrolyte composition were varied for type of acid, pH, type and amount of organic modifier based on analysis of protein standards. The HDL was separated from human serum by precipitation using Dextran sulfate, delipidated using 5% acetic acid, 5% acetonitrile, desalted using a 3k Centricon filter and re-suspended in buffer matching the corresponding background electrolyte. The CESI8000 system was fitted with a neutral surface capillary which was coupled to a Thermo Fusion mass spectrometer. Electrospray voltage was 1500 V and MS and MS/MS spectra were acquired at 60k and 30k resolution respectively. MS/MS was performed by selectively targeting specific charge states (isolation width m/z 5.0) from the protein standards and apolipoproteins to assess top down MS/MS quality.

PRELIMINARY DATA: Initial results showed that a background electrolyte of 10% acetic acid with 20% isopropanol provided the best separation of proteins. The resolving power was sufficient to enable deconvolution of protein ESI spectra to the observed molecular weight for some of the smaller proteins such as horse heart Apomyoglobin (17,083 Da), bovine carbonic anhydrase-II (29,114 Da), apolipoprotein A-II (17414.8 Da), Apo C-I (6630.6 Da), Apo C-II (8204.1 Da) and Apo C-III (8764.7 Da). Parameters for resolving larger proteins such as Apo A-I and Apo E have yet to be optimized. Many modified forms of the proteins were observed, consistent with the presence of truncated, oxidized and dimerized species, as shown in previous reports.

15:30 – 16:10

Dr. Bryan Fonslow
SCIEX, USA

CESI-MS Tips and Tricks

INTRODUCTION: Many widely-used separation methods and buffers have been modified to employ mass spectrometric (MS) detection since the additional power of MS generally outweighed the usage of traditional, non-MS compatible buffer additives. In particular, as capillary electrophoresis (CE) is being more widely used with MS, many of the traditional CE separation methods and background electrolytes (BGEs) are being replaced by common, MS-compatible, volatile buffers. In combination with volatile BGEs, the integration of CE and ESI into a single dynamic process (CESI) provides the capability of performing separations and detection with ultra-low flow rates, resulting in reduced ion suppression and improved sensitivity. We will describe many of the methodological considerations for CESI-MS of charged and polar molecules in different applications contexts.

METHODS: CESI-MS separations were achieved using OptiMS bare fused-silica, Neutral, and polyethyleneimine (PEI)-coated capillary cartridges by application of voltages from 30 kV to - 30 kV and low pressures (2 - 5 psi), resulting in flow rates from ~20 – 100 nL/min. Acetate-, formate-, and carbonate-based BGEs with isopropanol (IPA) and methanol (MeOH) additives were used as background electrolytes (BGE). Samples were simply diluted in 10% BGE, ammonium acetate, or up to 50% organic and injected electrokinetically using a voltage with field-amplified/enhanced sample injection (FASI/FESI) or with pressure followed by transient isotachophoresis (tITP) or field-amplified/enhanced sample stacking (FASS/FESS). Positive and negative ESI was performed with ~1 – 2 kV scanning anywhere from 50 m/z up to 15,000 m/z.

PRELIMINARY DATA: CESI-MS have proven to work on a wide range of molecules (~100 Da to ~1 MDa) with each application requiring specific experimental considerations. While applications for biologics, proteomics, metabolomics, glycomics, and various other areas have already been developed, general strategies can be employed to develop a CESI-MS method for your molecule of interest. Some considerations include the solubility; pKa, pKb, or pI; size; chirality, sample matrix; and the expected m/z of your molecule(s) of interest. A few application developments will be highlighted which showcase some of the steps used to create a new method. Analysis conditions can be optimized in a logical order to ensure the application is successful. CESI-MS best practices will also be described.

Day 2 – Novel & Advanced Applications and Biopharma

Time	Description
08:00 – 09:00	**Registration**
Novel & Advanced Applications | *Session Chair: Dr. Spencer Walse*	
09:00 – 09:40	**Applications of CESI-MS to Analytical Challenges in Agriculture** *Dr. Spencer Walse, USDA-ARS, USA*
09:40 – 10:20	**Integrated Capillary Electrophoresis and Electrospray Ionization (CESI) Mass Spectrometry for the Analysis of Polar Pesticide Residues in Tree Nuts and Comparison with Liquid Chromatography** *Dr. Wiley Hall, Safe Food Alliance, USA*
10:20 – 11:10	**Break – Posters**
11:10 – 11:50	**Status of Development of CESI-MS Methods and Hardware for Spaceflight Missions of Exploration** *Dr. Maria Fernanda Mora, Jet Propulsion Laboratory (JPL), California Institute of Technology, USA*
11:50 – 12:30	**Mass-spectrometry of Single Mammalian Cells Quantifies Proteome Heterogeneity During Cell Differentiation** *Professor Nikolai Slavov, Northeastern University, USA*
12:30 – 13:30	*Lunch*
Biopharma | *Session Chair: Professor John R. Yates III*	
13:30 – 14:10	**Comprehensive Analysis of HIV Glycosylation by Capillary Electrophoresis Separation and Ultraviolet Photodissociation** *Professor John R. Yates III, The Scripps Research Institute, USA*
14:10 – 14:50	**Characterization of Intact Glycoproteins by CESI-MS** *Dr. Elena Dominguez-Vega, Leiden University Medical Center, The Netherlands*
14:50 – 15:30	**Break – Posters**
15:30 – 16:10	**Middle-down, Top-down and Native CESI-MS in Characterization of Biopharmaceutical Proteins** *Arseniy Belov, Barnett Institute of Chemical & Biological Analysis, Department of Chemistry & Chemical Biology, Northeastern University*
16:10 – 16:50	**CESI-MS: A New Level of Separation for Charge Heterogeneity Analysis of Protein Therapeutics** *Dr. Esme Candish, SCIEX, USA*
16:50 – 17:20	**Break – Posters**
17:20 – 17:20	**CESI-MS Roundtable Sessions**
17:20 – 17:40	**Adjourn**
17:40 – 18:00	**Lab Tour** – *Sign-up during breaks*

09:00 – 09:40

Dr. Spencer Walse
USDA-ARS, USA

Dr. Spencer Walse,[1] Erik Rangel Rivera[2]
1 USDA-ARS, USA
2 Fresno State University, CA, USA

Applications of CESI-MS to Analytical Challenges in Agriculture

INTRODUCTION: Insects communicate with one another using a myriad of strategies, and the ability to control insect pests of agriculture in a sustainable manner is critical linked to the exploitation of this communication. Our understanding of chemical communication between insects has focused on the study of non-polar terpenoids, molecules generally amenable to gas chromatography and having minimal environmental reactivity, or at least, uninspiring aqueous chemistries. As recent applications of liquid chromatography have unveiled, it turns out these seemingly boring inter-insect terpenoid signals display fascinating conjugation chemistries and are, in fact, delivered as aqueous formulations into the environment. Here we highlight results of CESIMS analysis of the excreta of two key agricultural pests, the glassy-wing sharpshooter and the Mexican fruit fly.

METHODS: Experimental Hardware

Four HPLC-MS Instruments were used to collect data:

1. Thermo P4000 pump with Finnigan LCQ DecaXP Max
2. Shimadzu LC-10ADVP pump with Applied Biosystems Qstar-XL QTOF
3. Hewlett Packard 1090 pumping system with 5989 MS Engine and 59980 particle beam interface,
4. Agilent 1100 modular HPLC system with Agilent 5973 MSD and CSS Analytical Co Inc. Genesis II Particle Beam Interface, and an AB SCIEX CESI 8000 with 5600+ TripleTOF MS.

PRELIMINARY DATA: A diastereomeric mixture of the glucosylated pheromones (6R)- and (6S)-b-Dglucopyranosyl 2-(2,6-dimethyl-6-vinylcyclohex-1-enyl)acetate, respectively suspensoside A and suspensoside B, were isolated from the oral secretions of male Mexican fruit fly. HPLC - (-)ESIMS of suspensoside A and B (co-elution) over m/z 80-800 (A) and the corresponding ion trace of m/z 401 (B), MS2 spectra of m/z 401 [M + formate]- showing loss of formate (C), and MS3 spectra of the m/z (401®355) ions (D) that results from the carboxylate of the aglycon. HPLC - (+)ESIMS over m/z 80-800 (E), MS2 spectra of m/z 374 ([M + NH4]+) showing loss of a hexose monosaccharide (F), and MS3 spectra of the m/z (374®195) ions (G) that results from an acylium ion of the aglycon core 2,6-dimethyl-6-vinylcyclohex-1-ene acetic acid structure.

09:40 – 10:20

Dr. Wiley Hall
Safe Food Alliance, USA

Wiley A. Hall, 4th,[1] Spencer S. Walse,[2] Erik Rivera,[2] William C. Beckham,[1] and Thomas M. Jones[1]
1 Safe Food Alliance, USA
2 USDA-ARS, USA

Integrated CESI-MS for the Analysis of Polar Pesticide Residues in Tree Nuts and Comparison with Liquid Chromatography

ABSTRACT: Methods to quantitate residues of highly polar pesticides have been of great interest recently for a number of reasons including: highly publicized concerns about health effects (i.e. – glyphosate), MRL violations caused by biogenic and anthropogenic interferents (i.e. – fosetyl-aluminum, phosphonic acid and phosphoric acid), and just the large amounts of these compounds that are used each year (i.e. – glyphosate, glufosinate and paraquat. Analysis with LC-MSMS using stationary phases types such as: graphitized carbon, ion exchange, HILIC, mixed, and polar phases as well as IC-MSMS, have been reported, but these methods can have disadvantages such as matrix suppression, poor retention time stability (especially when analyzing residues in nut matrices), and a limited number of analytes (and analyte classes) that can be analyzed within a single method. There is no polar counterpart to the reversed phase multi-residue screens capable of testing for dozens to hundreds of compounds (for example, the QuPPe method for polar compounds has 8 separate analysis methods using at least 5 different LC columns).

Capillary electrophoresis, coupled to tandem mass spectrometry (CESI-MSMS), has been found to be a promising alternative to LC-MSMS analysis. Preliminary results from an inter-laboratory study using CESI- and LC-MSMS for the analysis of glyphosate, AMPA, glufosinate, fosetyl-Al, phosphonic and phosphoric acids, ethephon, paraquat, diquat, mepiquat, and chlormequat in tree nut matrices (< 10 minute separation time) show that show that CESI-MSMS can be highly effective in the separation of charged pesticides with highly stable retention times virtually no matrix suppression. The use of CESI-MSMS to elucidate the fate of phosphorous oxides (phosphite and phosphate) in agricultural products (fertilizers, fungicides, etc…) is also examined in comparison to LC-MSMS.

11:10 – 11:50

Dr. Maria Fernanda Mora
NASA Jet Propulsion Laboratory (JPL), USA

M. Mora,[1] F. Kehl,[1] J. Creamer,[1] E. Tavares da Costa,[1] A. Guttman,[2] B. Fonslow,[2] E. Candish,[2] J. Chapman,[2] and P. Willis[1]
1 Jet Propulsion Laboratory (JPL), California Institute of Technology, USA
2 SCIEX, USA

Status of Development of CESI-MS Methods and Hardware for Spaceflight Missions of Exploration

INTRODUCTION: The search for evidence of life beyond Earth is among the highest level goals in planetary exploration. However, despite multiple orbiter and landed missions to extraterrestrial bodies in the solar system, we still haven't found evidence of life. A powerful approach in this search involves seeking biochemical signatures of life at the molecular level, as distributions of organic molecules. The separation techniques capillary electrophoresis (CE) and its miniaturized version, microchip electrophoresis (ME) overcome the limitations of gas-phase techniques and hold unique promise in the search for signatures of life on other worlds. Here we describe the status of instrumentation developed at JPL and the steps we are taking to someday enable the its implementation on other worlds.

METHODS: Although multiple detection methods can be coupled to CE and ME, we focused on the two most powerful detection techniques: mass spectrometry (MS) and laser-induced fluorescence (LIF). LIF offers the highest sensitivity to organics while MS allows complete identification. These techniques are complementary of each other and would allow full characterization of a sample in situ.

We describe here The Chemical Laptop, a portable ME-LIF system which would provide the sample processing capabilities for in situ analysis with ppb sensitivity and the Organic Capillary Electrophoresis Analysis System (OCEANS) that couples CE with electrospray ionization MS (CESI-MS), in order to enable the characterization of distributions of organics on future in situ ocean worlds missions.

PRELIMINARY DATA: The Chemical Laptop is the first battery-powered and truly portable "end-to-end" ME-LIF astrobiology instrument capable of receiving an unlabeled liquid sample and performing all operations required for ME-LIF analysis. The search for signatures of life on alien worlds imposes a low sensitivity requirement on in situ instrumentation. Toward this end, we have optimized the optical system on the Chemical Laptop to achieve extremely low instrument detection limits. We recently validated The Chemical Laptop by analyzing amino acids extracted from the soil in the Atacama Desert in Chile.

Regarding OCEANS, we are building a capillary-based system including a new injector and sample handling system. We are performing vibration tests of the most fragile components (such as the CESI porous tip) in order to increase the TRL of the system so it's ready for flight. We are also developing separation methods for amino acids that are tolerant to high salt concentrations as the ones expected to be found on the surface of Europa. These methods involve tITP to increase the sensitivity to amino acids. We are also currently testing buffer systems that are more compatible with flight than the most common CESI-MS buffers acetic acid and formic acid.

11:50 – 12:30

Professor Nikolai Slavov
Northeastern University, USA

Bogdan Budnik,[1] Ezra Levy,[2] and Nikolai Slavov[3]
1 MSPRL, FAS Division of Science, Harvard University, Cambridge, MA 02138, USA
2 Department of Biology, Northeastern University, Boston, MA 02115, USA
3 Department of Bioengineering, Northeastern University, Boston, MA 02115, USA

Mass-spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation

INTRODUCTION: Cellular systems, such as tissues, cancers, and cell cultures, consist of a variety of cells with distinct molecular and functional properties. Characterizing such cellular differences is key to understanding normal physiology, combating cancer recurrence, and enhancing targeted differentiation for regenerative therapies; it demands quantifying the proteomes of single cells.

METHODS: To quantify the proteomes of single mammalian cells, we resolved two major challenges: (i) delivering the proteome of a mammalian cell to a MS instrument with minimal protein losses and (ii) simultaneously identifying and quantifying peptides from single cell samples. To overcome the first challenge, we manually picked live single cells under a microscope and lysed them mechanically (by sonication in glass microtubes) in phosphate buffered saline. To overcome the second challenge, we made novel use of tandem mass tags (TMT) and their ability to quantify the level of each TMT labeled peptide in each sample while identifying its sequence from the total peptide amount pooled across all samples.

PRELIMINARY DATA: Cellular heterogeneity is important to biological processes, including cancer and development. However, proteome heterogeneity is largely unexplored because of the limitations of existing methods for quantifying protein levels in single cells. To alleviate these limitations, we developed Single Cell ProtEomics by Mass Spectrometry (SCoPE-MS), and validated its ability to identify distinct human cancer cell types based on their proteomes. We used SCoPE-MS to quantify over a thousand proteins in differentiating mouse embryonic stem (ES) cells. The single-cell proteomes enabled us to deconstruct cell populations and infer protein abundance relationships. Comparison between single-cell proteomes and transcriptomes indicated coordinated mRNA and protein covariation. Yet many genes exhibited functionally concerted and distinct regulatory patterns at the mRNA and the protein levels, suggesting that post-transcriptional regulatory mechanisms contribute to proteome remodeling during lineage specification, especially for developmental genes. SCoPE-MS is broadly applicable to measuring proteome configurations of single cells and linking them to functional phenotypes, such as cell type and differentiation potentials.

13:30 – 14:10

Professor John R. Yates III
The Scripps Research Institute, USA

John R. Yates III[1] and Jolene Diedrich[1,2]
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Comprehensive Analysis of HIV Glycosylation by Capillary Electrophoresis Separation and Ultraviolet Photodissociation

INTRODUCTION: HIV-1 envelope glycoprotein (Env) is the sole target for neutralizing antibodies and is the focus for design of an HIV vaccine. The Env trimer is covered by ~90 N-linked glycans that shield the underlying protein from immune surveillance. Yet broadly neutralizing antibodies of HIV are found, and many show dependence on glycans for binding to Env. The relevance of glycosylation to vaccine design has underscored the need to routinely assess the glycan type at each glycosylation site.

METHODS: We demonstrate glycopeptide analysis on a hybrid quadrupole mass filter/linear ion trap/Orbitrap mass spectrometer, which has been modified to allow ultraviolet photodissociation (UVPD) ion fragmentation. CESI (Capillary Electrophoresis with Electrospray Ionization) and reverse phase UPLC are utilized for peptide separation directly coupled to the mass spectrometer.

PRELIMINARY DATA: Multiple proteolytic enzymes were used to digest the HIV-1 protein gp120 to maximize sequence coverage and achieve nearly complete sequence coverage. We demonstrate methods and respective data for approaches that either remove glycans prior to analysis or retain them during LCMS. Peptides samples were analyzed on the Thermo Fusion Tribrid Orbitrap and Thermo Lumos Fusion Tribrid Orbitrap Mass Spectrometers utilizing separation by either CESI or reversed phase UPLC. A variety of fragmentation techniques are discussed, including CID, HCD, ETD, EThcD, and UVPD. We also demonstrate separations by CESI that can achieve very low flow rates and excellent separation of the glycopeptides.

NOVEL ASPECT: CESI with UVPD fragmentation for glycoprotein analysis

14:10 – 14:50

Dr. Elena Dominguez-Vega
Leiden University Medical Center, The Netherlands

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Characterization of Intact Glycoproteins by CESI-MS

INTRODUCTION: Glycosylation is one of the most common posttranslational modifications of proteins and it is involved in a wide range of biological process. Alteration of glycosylation has been associated with numerous diseases, including inflammation and cancer. Furthermore, most of the proteins exploited nowadays by the pharmaceutical industry are glycosylated. These glycan decorations have a major impact on the efficacy and half-life of the therapeutic protein, and glycosylation is considered one of its critical quality attributes. Common methods to study glycosylation involve glycan release or protein digestion and analysis of the resulted glycans or glycopeptides. Analysis at the intact protein level offers complementary information and it allows identification of co-occurring modifications.

METHODS: Sheathless integrated capillary electrophoresis electrospray ionization (CESI) was carried out on a CESI 8000 instrument (Sciex, Brea, CA) coupled to either a MAXIS HD or an Impact qTOF mass spectrometer from Bruker Daltonics equipped with a nano-electrospray source. CESI-MS of proteins was performed using in-house polyethylenimine (PEI) coated capillaries or commercially-available neutrally-coated capillaries (Neutral OptiMS Cartridge, Sciex) with a porous tip. Solutions of 1-20% acetic acid containing 0-5% isopropanol were used as background electrolytes.

PRELIMINARY DATA: This study shows the power of CESI-MS for the assessment of protein glycosylation of intact proteins relevant to pharmaceutical and biomedical sciences. Several CESI-MS approaches, including intact and middle-up level of analysis, have been developed and applied to various glycoproteins exhibiting different glycan complexity and number of glycosylation sites. To prevent protein adsorption, we employed dedicated charged and neutrally-coated capillaries providing efficient protein separation. The developed CESI-MS methods exhibit the selectivity and sensitivity needed to reliably detect the existing glycoforms. Next to glycosylation, other co-occurring protein modifications, including deamidation and truncation, were revealed. In this presentation, the performance of the CESI-MS systems will be highlighted with focus on the analysis of proteins of pharmaceutical and clinical interest, such as antibody-derived therapeutics, prostate-specific antigen (PSA) and serum IgG Fc portions.

15:30 – 16:10

Arseniy Belov

Barnett Institute of Chemical & Biological Analysis, Department of Chemistry & Chemical Biology, Northeastern University, USA

Arseniy M. Belov,[1] David R. Bush,[2] Li Zang,[3] Marcia R. Santos,[4] Rosa Viner,[5] Barry L. Karger,[1] and Alexander R. Ivanov[1]

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Middle-Down, Top-Down, and Native CESI-MS in the Characterization of Biopharmaceutical Proteins

ABSTRACT: A conventional electrospray ionization mass spectrometry (ESI-MS)-based approach for the analysis of biopharmaceutical proteins involves bottom-up proteomics. However, as proteins are digested into peptides, proteoforms and their quantities may not be readily determined. Alternative approaches involve top-down proteomics, where individual proteoforms are characterized directly, or middle-down proteomics, involving the characterization of large fragments following limited proteolysis. An extension of top-down proteomics is native MS, involving protein analysis at near physiological conditions when non-covalent interactions and labile post-translational modification are preserved. However, protein complexity necessitates high resolution separations prior to ESI-MS. While liquid chromatography has been used most often for protein and peptide separations, capillary zone electrophoresis (CZE) offers a complementary approach.

METHODS: In this work, three biopharmaceutical proteins were characterized by top-down, middle-down, or native CZE-MS approaches using capillaries with covalent coatings coupled online to Orbitrap mass spectrometers via the CESI interface. First, recombinant human interferon-β1 (Avonex) was analyzed in a top-down approach by CZE-MS using a cross-linked polyethylenimine-coated capillary coupled to the Orbitrap Elite. Next, top-down and middle-down approaches were used for the characterization of two different biopharmaceutical monoclonal antibodies (mAbs) by CZE-MS on a capillary with a positively-charged coating (M7C4I) coupled to the QExactive Plus. The middle-down analysis was achieved by digestion of the mAb with IdeS protease. These results were complemented by native CZE-MS analyses on a system composed of a polyacrylamide-coated capillary coupled to Orbitrap mass spectrometers.

PRELIMINARY DATA: Polyethylenimine-coated capillary was coupled to the Orbitrap Elite for the characterization of recombinant human interferon β-1 in a top-down approach by CZEMS, resulting in the separation of proteoforms due to sialyation, deamidation, and 2 glycosylation. In total, 138 individual proteoforms were identified and 55 were quantitated.

Next, a biopharmaceutical mAb was analyzed by intact CZE-MS, resulting in the qualitative and quantitative characterization of glycoforms. Intact analysis was performed on a 1-(4-iodobutyl)4-aza-1-azoniabicyclo[2.2.2]octane

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iodide (M7C4I)-coated capillary coupled to the QExactive Plus and resulted in the separation of 2X-glycosylated, 1X-glycosylated, and aglycosylated populations of the mAb due to hydrodynamic volume differences. Intact analysis allowed for the detection of species corresponding to the mAb with two dissociated light chains, thus suggesting that a trace amount of the mAb is composed of individual chains held together by non-covalent forces instead of disulfide bonds. These results were complemented by CZE-MS analysis under native conditions on a polyacrylamide-coated capillary coupled to the QExactive Plus, resulting in validation of the determined relative abundances of intact proteoforms.

Intact and native CZE-MS results were followed by middle-down analysis of the mAb on a M7C4I-coated capillary coupled to the QExactive Plus, resulting in the identification of additional proteoforms that could not be observed in intact/native approaches. Moreover, baseline separation of deamidated proteoforms due to charge variation and the separation of glycoforms based on differences in hydrodynamic volume were obtained.

A different monoclonal antibody was analyzed under denaturing conditions on the M7C4Icoated capillary coupled to the QExactive Plus, resulting in the identification of 2X- and 1Xglycosylated populations and species corresponding to the mAb with two dissociated light chains. These results were validated using native CZE-MS performed on a polyacrylamide-coated capillary coupled to the Exactive EMR. Native CZE-MS analysis also resulted in the identification of dimers at a signal intensity of approximately 1%.

16:10 – 16:50

Dr. Esme Candish
SCIEX, USA

Esme Candish,[1] Christopher Loessner,[2] Steve Lock,[3] and Bryan Fonslow[1]
1 SCIEX, USA
2 SCIEX, Germany
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CESI-MS: A New Level of Separation for Charge Heterogeneity Analysis of Protein Therapeutics

INTRODUCTION: Introduction: Therapeutic mAbs can exhibit significant microheterogeneity due to the numerous post-translational modifications (PTMs), sequence variants, and degradation products that occur during production and storage. Critical quality attributes, such as deamidation, oxidation, and glycosylation can affect their efficacy, bioavailability, and biosafety. Therefore, the comprehensive characterization of therapeutic mAbs is essential for both product development and process control. High resolution separations of intact mAbs, such as capillary electrophoresis (CE), prior to MS detection may facilitate the detection of smaller mass modifications, such as deamidation (0.984 Da). The integration of CE and electrospray ionization (ESI) into one dynamic process (CESI) has many benefits and applications. In this case, we demonstrate CESI-MS for the comprehensive, rapid and robust characterization of intact mAb charge variants.

METHODS: IgGs were either diluted directly from the formulation buffer or desalted directly into the sample buffer for stacking. Rapid CE separations of IgG charge variants were achieved using a SCIEX CESI cartridge with a positive polyethyleneimine (PEI) surface coating. MS compatible background electrolytes (BGEs) comprised of acetic acid facilitated highly efficient separations, achieved in less than 15 mins. CESI analyses were performed using a SCIEX CESI 8000 Plus directly coupled to a SCIEX TripleTOF® 6600 which scanned over a mass range of 2000-4000 m/z. Data were analyzed using SCIEX PeakView® software and the SCIEX BioPharmaViewTM software.

PRELIMINARY DATA: PTMs and degradation products often alter the net charge of therapeutic mAbs, consequently changing the electrophoretic mobility of the modified products. The change in net charge due to these modifications was exploited to separate these therapeutic mAbs variants. Directly coupling CE with MS using the CESI-MS platform provides a powerful and streamlined tool that enables charge variants to be separated and identified in a single analysis. The positively charged PEI surface coating provided an electroosmotic flow (EOF) of approximately 2 mm/s which permitted the separation of all therapeutic intact mAbs in less than 15 min. Despite the high EOF, the 90 cm long separation capillary facilitated resolution in the CE domain. Each of the therapeutic mAbs analyzed by CESI-MS revealed several charge variants in the separation profile. For example, the representative mAb Trastuzumab (n=3, originator material), revealed the near baseline separation of at least three dominate charge variants. A very high run-to-run repeatability was achieved for this assay with the migration time percent relative standard deviations (%RSD) of just 0.13, 0.14 and 0.16% for the three charge variants respectively. The low deviation in the migration times highlighted the robustness of the method.

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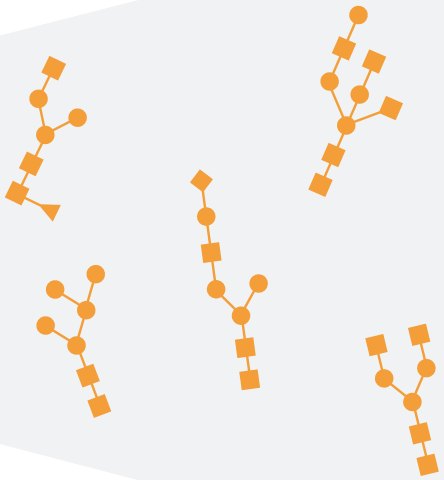
By coupling CESI with a Q-TOF-MS we were able to achieve information-rich, reproducible mass spectra for intact mAbs. Mass spectral deconvolution of each peak revealed that charge variants were attributed to mass shifts of as little as +1 Da, likely from known asparagine deamination on Trastuzumab. The small mass increase associated with this degradation product of mAbs has made it notoriously difficult to characterize and assign at the intact level. The ability of CESI-MS to resolve deamidation products of intact mAbs, and therefore characterize acidic charge variants not possible by MS alone, is a particularly exciting outcome of this work.

NOVEL ASPECT: Exploiting the power of CE charge variant separations coupled with MS to improve the comprehensive characterization of intact therapeutic mAbs

See how

CESI-MS



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