2016

Inaugural Global CESI-MS Symposium

Final Program and Abstract Book

VU University | Amsterdam, The Netherlands | October 6-7, 2016

2016

Inaugural Global CESI-MS Symposium

VU University | Amsterdam, The Netherlands | October 6-7, 2016

Symposium Chair

Professor Govert Somsen
VU University Amsterdam, The Netherlands

Sponsored by SCIEX

DAY ONE – Biopharmaceuticals and Glycopeptidomics	
Time	Description
09:00 – 11.00	Registration
11:00 – 11:10	**Professor Govert Somsen**, VU University Amsterdam, The Netherlands *Introduction & Chair*
11:10 – 11:50	**Dr. Rob Haselberg**, VU University Amsterdam, The Netherlands *CESI-MS Practicalities from Separation to Detection*
11:50 – 12:30	**Professor Emmanuelle Leize**, University of Strasbourg, France *CESI-MS Coupling for the Detailed Characterization of Therapeutic Proteins and Their Biological Complexes*
12:30 – 13:30	Lunch and poster session
13:30 – 14:10	**Dr. Elena Dominguez Vega**, VU University Amsterdam, The Netherlands *Intact Proteoform Characterization Using CESI–MS*
14:10 – 14:50	**Dr. Yoann Ladner**, Institut des Biomolécules Max Mousseron, Montpellier, France *CESI-MS developments for the analysis of anti-TNFα and their Anti-Drug Antibodies (ADA)*
14:50 – 15:10	Coffee Break
15:10 – 15:50	**Professor Andras Guttman**, University of Pannonia, Hungary *Characterization of N-Linked Carbohydrates in CE-LIF and CESI-MS Modes*
15:50 – 16:30	**Guinevere Kammeijer**, Leiden University Medical Center, The Netherlands *Unravelling the Bitter Sweetness of Prostate Specific Antigen with CESI-MS*
16:30 – 17:20	KEYNOTE PRESENTATION **Professor John Yates III**, The Scripps Research Institute, USA *CEMS for Top Down of Proteins Complexes and Beyond*
17:20 – 17:30	**Professor Govert Somsen**, VU University Amsterdam, The Netherlands *Concluding Remarks – Day One*
18:30 – 22:00	**Conference Excursion** – *SCIEX-sponsored boat trip through the Amsterdam canals*

11:00 – 11:10

Dr. Rob Haselberg
VU University Amsterdam, The Netherlands

CE-MS practicalities from separation to detection

ABSTRACT: Throughout the symposium many examples of the added value of using the combination of capillary electrophoresis and mass spectrometry (CE-MS) will be highlighted. However, before these results can be obtained suitable and robust methods have to be developed. Therefore, in this lecture practicalities of CE-MS method development are discussed. CE-MS can be performed in a robust way, as will be exemplified with results from the first global CE-MS interlaboratory study. Next, factors that can affect the performance of a method will be treated. This will systematically go from separation conditions, via interfacing parameters to mass spectrometric detection settings. Comparisons in terms of performance between different CE-MS systems will be treated as well. Last, some insights in how increase the sample load and throughput will be provided.

Notes:

11:50 – 12:30

Professor Emmanuelle Leize
University of Strasbourg, France

CESI-MS Coupling for the Detailed Characterization of Therapeutic Proteins and Their Biological Complexes

ABSTRACT: Protein characterization represents very challenging questions to the analytical sciences, in particular full protein characterization as now requested by regulation agencies. In this context, the LSMIS develops well-adapted Capillary Electrophoresis-Mass Spectrometry (CESI-MS) coupling-based approaches for the detailed characterization of proteins and their biological complexes (collaboration with Sciex Separation). In the case of therapeutic proteins, CESI-MS enables for the first time the access, in a single injection and with less than one picomole of protein, to the complete structure (including glycosylations/modifications) of several monoclonal antibodies (mAbs) and their related products (biosimilars, Antibody-Drug Conjugates).

Notes:

13:30 – 14:10

Dr. Elena Dominguez Vega
VU University Amsterdam, The Netherlands

Rob Haselberg, Govert W. Somsen,
Division of BioAnalytical Chemistry, Vrije Universiteit Amsterdam, The Netherlands, e.dominguezvega@vu.nl

Intact Proteoform Characterization Using CESI–MS

ABSTRACT: The identification and quantification of intact proteins by mass spectrometry (MS) is an emergent technology. While attainable mass resolution and accuracy are steadily advancing, sample complexity remains a challenge for intact protein analysis by MS. Even isolated or manufactured proteins, such as biopharmaceuticals, often encompass highly similar proteoforms which cannot be distinguished consistently by MS only. Separation prior to MS analysis is required to achieve reliable and sensitive characterization of (minor) protein variants and modifications. However, efficient separation of intact proteoforms under MS-compatible conditions is not straightforward.

Sheathless integrated capillary electrophoresis electrospray ionization (CESI) offers attractive possibilities for mass spectrometric (MS) detection of intact proteins. It provides the selectivity and sensitivity required to distinguish closely-related protein variants and isoforms. We have developed several CESI-MS approaches for the assessment of the (micro) heterogeneity of intact protein samples revealing detailed differences in e.g. deamidation, glycosylation, truncation and conformation. Examples will highlight the CESI-MS performance with focus on the intact and middle-up analysis of proteins of pharmaceutical and biomedical interest, such as the amyloidogenic protein beta-2-microglobulin, single-domain antibody preparations ('nanobodies') targeting GPCR receptors or monoclonal antibodies (mAbs).

Notes:

14:10 – 14:50

Dr. Yoann Ladner

Institut des Biomolécules Max Mousseron, Montpellier, France

Yoann Ladner[1], Gaëlle Coussot[1], Silvia Mas[1], Christian Larroque[2], Pierre Martineau[2], Pierre-Antoine Bonnet[1], André Pèlegrin[2], Jacques Morel[3], Catherine Perrin[1]

1 Institut des Biomolécules Max Mousseron, UMR 5247, Faculté de Pharmacie, 34093 Montpellier cedex 5, France

2 Institut de Recherche en Cancérologie de Montpellier, Campus Val d'Aurèle, 34298 Montpellier cedex 5, France

3 Centre Hospitalier Universitaire & Université de Montpellier, 34295 Montpellier cedex 5, France

CESI-MS developments for the analysis of anti-TNFα and their Anti-Drug Antibodies (ADA)

ABSTRACT: Therapeutic monoclonal antibodies (TmAbs) constitute an active and expanding pharmaceutical class. As biological protein derivatives, TmAbs are subjects to induce immunological reactions, causal factors of potentially serious adverse effects or limiting their clinical potency. These effects could be explained by the formation of anti-drug antibodies (ADA).

The measurement of ADA levels and the monitoring of the development of immunogenicity give important information on the efficiency of the treatment and the possible induction of tolerance. Currently used methods for ADA detection are all immunologically based including diverse ELISA methods and radioimmunoassays such as the antigen-binding test (ABT) or assay (ABA)[1].

Although essential, those methods are known to have limitations since the level of the TmAb in patients'sera remain a complicating factor for appropriate measurement[2]. Consequently, the arsenal of methods for ADA determination and assay is nowadays clearly insufficient. Considering the potential of adverse effects and limitations in mid- or long-term therapeutic objectives, development of novel strategies for analytical measurements is a priority[3]. The development of biologically appropriate separation methods for physicochemical characterization of bioactive molecules appears to offer new possibilities in the monitoring of drug effects.

In this context, our consortium is currently involved in a project[4] aiming at developing capillary electrophoresis associated with mass spectrometry (CESI-MS) methodologies complementary to ELISA and pH-shift anti-idiotype antigene-binding (PIA) tests for a better understanding and follow up of the development of immunogenicity with the use of therapeutic monoclonal antibodies (TmAbs)as reference bioassays. Five anti-TNF biological drugs used in the treatment of rheumatoid arthritis have been selected as reference molecules for this project: infliximab, adalimumab, certolizumab pegol, golimumab and etanercept.

Primary CESI-Ms results for (1) the control of antibody formulations and (2) the analysis of drugs and their ADA on a diversity of serum samples of patients treated for RA will be presented. Sample preparation strategies will be also detailed to allow the ADA characterization.

1. Aarden L., Ruuls S.R., Wolbink G., Immunogenicity of anti-tumor necrosis factor antibodies- toward improved methods of anti-antibody measurement, *Current Opinion in Immunology* 2008; 20: 431-435.
2. Lofgren J.A., Wala I., Koren E., Swanson S.J., Jing S., Detection of neutralizing anti-therapeutic protein antibodies in serum or plasma samples containing high levels of the therapeutic protein, *Journal of Immunological Methods* 2006; 308: 101-108.
3. Shuptrine C.W., Surana R., Weiner L.M., Monoclonal antibodies for the treatment of cancer. *Seminars in Cancer Biology* 2012; 22: 3-13.
4. Project "Therapeutic monoclonal antibodies and immunogenicity: Development of analytical methods for anti-TNF therapy", supported by the French Agency for the Safety of Drugs and other Health Products (ANSM)

15:10 – 15:50

Professor Andras Guttman, *University of Pannonia, Hungary*
Andras Guttman[1,2], Bryan Fonslow[1,3], and Marton Szigeti[2]
1 SCIEX, Brea, CA, USA
2 Horvath Csaba Laboratory of Bioseparation Sciences, University of Debrecen, Hungary,
3 The Scripps Research Institute, La Jolla, CA, USA

Characterization of N-Linked Carbohydrates in CE-LIF and CESI-MS Modes: The versatility of the CESI 8000 system

ABSTRACT: Characterization of the N-linked glycosylation of therapeutic glycoproteins is routinely done by capillary electrophoresis - laser induced fluorescent detection (CE-LIF) after endoglycosidase based carbohydrate release and aminopyrenetrisulfonate (APTS) tagging. CE separation provides high resolution and migration time-based identification of glycans using relevant GU databases, but coupling with MS detection offers additional structural verification. Integration of CE and electrospray ionization (ESI) into a single dynamic process provides the capability of performing CE separation and MS ionization with ultra-low flow rates, resulting in reduced ion suppression and improved sensitivity. However, the transition from current CE-LIF APTS-glycan analysis methods to CESI-MS requires the use of MS-compatible buffers. High resolution was obtained with the use of low pH ammonium acetate buffer with 10% isopropanol. Our results revealed that in negative ionization mode using reversed CE polarity (and consequently negative EOF) and low forward pressure resulted in excellent separation conditions to obtain high efficiency. Using the optimized method (voltage and pressure) and buffer system, APTS-labeled monoclonal antibody N-glycans were efficiently analyzed in negative MS mode without risking in-source sugar residue rearrangement.

Notes:

15:50 – 16:30

Guinevere Kammeijer

Leiden University Medical Center, The Netherlands

Guinevere S.M. Kammeijer[1], Jan Nouta1, Theo M. de Reijke[2], Jean J.M.C.H. de la Rosette[2], Manfred Wuhrer[1]

1 Leiden University Medical Center, Center for Proteomics and Metabolomics, Leiden, The Netherlands

2 Academic Medical Center, Department of Urology, University of Amsterdam, Amsterdam, The Netherlands

Unravelling the Bitter Sweetness of Prostate Specific Antigen with CESI-MS

ABSTRACT: In 2012 more than a million new prostate cancer (PCa) cases were diagnosed worldwide, with PCa being the most prevailing cancer in men after lung cancer.[1] Since 1994, the protein concentration of prostate specific antigen (PSA) in serum is approved by the FDA as an early screening method of PCa. However, recent studies revealed that the test lacks specificity as increased PSA concentrations are not only caused by PCa. For example, an enlarged prostate (benign hyperplasia, BPH) or inflammation of the prostate (prostatitis) can also result in elevated levels of PSA. Next to that, the PSA test is not capable to distinguish between a malignant or benign tumor, resulting in unnecessary biopsies. Therefore, there is an urgent need for a more specific biomarker. Literature shows that glycosylation of PSA could be used for a more defined test.[2-4] Nonetheless, a detailed analysis of the molecular features of PSA glycosylation (e.g., antennae decoration and core fucosylation) and their potential to improve the PCa diagnosis has not yet been assessed.

This study takes advantage of the isomeric separation of sialic acids at a low pH using capillary electrophoresis (CE) hyphenated to a quadruple time of flight (Q-TOF) mass spectrometer (MS) with electrospray ionization (ESI). As only minor amounts of PSA are found in urine, with median values of 103 µg/L for healthy volunteers, 206 µg/L for BPH patients and 425 µg/L for PCa patients,[5] a sheathless interface (CESI) with a dopant enriched nitrogen gas (DEN-gas) was used, which revealed an improved sensitivity and repeatability for glycopeptide analysis when compared to CESI without the usage of DEN-gas.[6] Furthermore, a PSA glycomic assay (PGA) was established which captures PSA from urine, followed by a tryptic digestion and a measurement on the CESI-MS system. The PGA was validated by monitoring the glycopeptide profile of PSA spiked female urine. After verification, the PGA was used to examine the glycosylation patterns of PSA in urine of PCa patients.

Overall, the preliminary data regarding the PGA showed promising results and will be further investigated for its potential to identify glycosylation alterations in PSA specifically related to PCa or BPH. This research pursues an enhanced assessment for future prognostic and diagnostic predictions.

1. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D. M.; Forman, D.; Bray, F. *Int. J. Cancer* 2015, 136, E359-E386.
2. Jankovic, M. M.; Kosanovic, M. M. Clin. Biochem. 2005, 38, 58-65.
3. Peracaula, R.; Tabarés, G.; Royle, L.; Harvey, D. J.; Dwek, R. A.; Rudd, P. M.; de Llorens, R. *Glycobiology* 2003, 13, 457-470.
4. Saldova, R.; Fan, Y.; Fitzpatrick, J. M.; Watson, R. W. G.; Rudd, P. M. Glycobiology 2011, 21, 195-205.
5. Vermassen, T.; Van Praet, C.; Vanderschaeghe, D.; Maenhout, T.; Lumen, N.; Callewaert, N.; Hoebeke, P.; Van Belle, S.; Rottey, S.; Delanghe, *J. Electrophoresis* 2014, 35, 1017-1024.
6. Kammeijer, G. S. M.; Kohler, I.; Jansen, B. C.; Hensbergen, P. J.; Mayboroda, O. A.; Falck, D.; Wuhrer, M. *Anal. Chem.* 2016, 88, 5849-5856.

16:30 – 17:20

Professor John Yates III,
The Scripps Research Institute, USA

Yates, III Jr, Wang Y., Fonslow B., Diedrich, J., Han, X., Lavallee-Adam, M., Moresco, J.
Chemical Physiology, 10550 North Torrey Pines Road, SR11, The Scripps Research Institute, LaJolla, CA 92037

Keynote Presentation

CEMS for Top Down of Proteins Complexes and Beyond

ABSTRACT: A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large-scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale analysis of protein data from experiments. Advances in multi-dimensional separations as well as mass spectrometry have improved the scale of experiments for protein identification. We will show recent data from a 2-D CE system for large-scale proteomics studies. We will also show analysis of protein complexes using CE methods with the ultimate goal of better analysis of proteoforms of the components of the complex. Recent data from Ultraviolet Photodissociation of proteins together with new informatics approaches will be shown.

Notes:

DAY TWO – Omics Research	
Time	Description
09:00 – 09:30	Registration
09:30 – 09:40	**Professor Manfred Wuhrer**, Leiden University Medical Center, The Netherlands *Introduction & Chair*
09:40 – 10:20	**Dr. Rawi Ramautar**, Leiden Academic Center for Drug Research, The Netherlands *CESI-MS: A Gateway to New Metabolomics Applications*
10:20 – 11:00	**Dr. Xavier Meniche**, Bioaster, Lyon, France *Identification of Short Chain Fatty Acids by CESI-MS Using a Neutral Capillary in Negative Mode*
11:00 – 11:20	Coffee Break
11:20 – 12:00	**Dr. Wiley Hall**, Dried Fruit Association of California, USA *A Comparison of the Challenges in the Quantification of Tree Nut Residues of Phosphonic Acid and Its Salts with CESI-MSMS and LC-MSMS.*
12:00 – 12:40	**Dr. Nicolas Heinzel**, Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany *The Detection of Plant Metabolites Using CESI-MS*
12:40 – 13:20	**Dr. Andreas Schmidt**, Ludwig-Maximilians-University of Munich, Germany *CESI-MS in Combination with SWATH™ Acquisition for the Analysis of Tryptic Digests*
13:20 – 14:00	Lunch and Poster Session
14:00 – 14:40	**Dr. Stephen Lock**, SCIEX, UK *Peptide Quantitation by CESI-MS.*
14:40 – 15:20	**Professor Alexander R. Ivanov**, Barnett Institute of Chemical and Biological Analysis, USA *Analysis of Biopharmaceutical Proteins, Protein Complexes and Proteomes Using CESI-MS*
15:20 – 16:10	KEYNOTE PRESENTATION **Professor Jennifer Van Eyk**, Cedars-Sinai Medical Center, USA *Analysis of Disease-Induced Protein Modifications Using CESI-MS: A Twist to Increase Disease Specificities*
16:10 – 16:20	**Professor Manfred Wuhrer**, Leiden University Medical Center, The Netherlands *Concluding Remarks — Day Two*

09:40 – 10:20

Dr. Rawi Ramautar

1 Leiden Academic Center for Drug Research, Leiden University, Leiden, The Netherlands

** Corresponding author: r.ramautar@lacdr.leidenuniv.nl*

CESI-MS: A Gateway to New Metabolomics Applications

ABSTRACT: In the field of metabolomics, a major challenge is the analysis of highly polar and charged metabolites in complex sample matrices. Here we demonstrate the utility of CESI-MS for the profiling of ionogenic metabolites in biological samples. The intrinsically low-flow characteristics of CE are effectively utilized in this set-up, enabling ESI-MS to reach its optimal performance, that is, an improved sensitivity and reduced ion suppression. As a consequence, CESI-MS allows the highly sensitive profiling of a broad array of metabolites in biological samples. The applicability is especially shown for the analysis of highly polar ionogenic metabolites, including structural isomers and isobaric compounds, for which a highly efficient and selective analytical method is urgently needed in metabolomics.

The use of CESI-MS under low-flow separation conditions with an injection volume of only ca. 20 nanoliter resulted in nanomolar detection limits for a broad array of highly polar metabolite classes, such as sugar phosphates, organic acids and nucleotides. Structural isomers of phosphorylated sugars as well as isobaric metabolites could be selectively analyzed by CESI-MS without using any derivatization. A unique feature of the proposed approach is that it allows the profiling of both anionic and cationic metabolites using a single capillary/buffer combination by only switching the MS detection and separation voltage polarity. The utility of CESI-MS for metabolic profiling of biological samples was investigated for mouse CSF, of which only a few microliters can be obtained under proper experimental conditions, and for samples containing only a limited number of cells. It is shown that highly information-rich metabolic profiles could be obtained for these limited amounts of biological samples. For example, approximately 300 molecular features (S/N>10) were detected in mouse CSF, whereas about 100 molecular features (S/N>5) were found in a glioblastoma cell line extract with a cell density of 20 cells/nL by CESI-MS.

Overall, CESI-MS will allow important metabolomics studies that have so far been lacking and it will open ways for a deeper understanding of biological functions in sample-limited cases.

Notes:

10:20 – 11:00

Dr. Xavier Meniche
Bioaster, Lyon, France

Identification of Short Chain Fatty Acids by CESI-MS Using a Neutral Capillary in Negative Mode

ABSTRACT: Short chain fatty acids (SCFA) play an important role in the health of the human intestine. These organic acids are the end product of carbohydrates fermentation by the gut microbiota. It has been shown that they are involved in defense mechanisms that inhibit infection of the gut by pathogenic bacteria (e.g. shigella). Indeed, SCFA induce production of antimicrobial peptides by the epithelial cells of the intestine and inhibits the activity of bacterial toxins like Stx2 secreted by *E. coli* EHEC[1]. The aim of this project is to develop a method using the CESI-MS to identify and quantify SCFA in the gut and to distinguish a "healthy" versus a "non-healthy" microbiota. SCFA are polar acidic molecules that are soluble in buffer containing organic solvents. First, we tried to develop a method to separate SCFA with the standard fused-silica capillary using reverse polarity. Although we tested several different conditions, we did not detect the SCFA assuming that it was because the EOF was higher than the electrophoretic mobility of the molecules. Thus, to suppress the effect of the EOF, we tested the newly commercialized neutral capillary, and we obtained very promising results.

1. Ashida H et al., (2011), *Nature Chemical Biology,* 8(1): 36-45

Notes:

11:20 – 12:00

Dr. Wiley Hall
Dried Fruit Association of California, USA

A Comparison of the Challenges in the Quantification of Tree Nut Residues of Phosphonic Acid and Its Salts with CESI-MSMS and LC-MSMS.

ABSTRACT: The export of tree nuts to the European Union (EU) is subject to a maximum residue limit (MRL) for the fungicide fosetyl-Al. As fosetyl-Al hydrolyzes readily to yield phosphonic acid, the residue of fosetyl-Al is quantitated as the *"sum of fosetyl, phosphonic acid, and their salts"*. Unfortunately, phosphonate in tree nut extracts can originate from several anthropogenic and biogenic sources besides fosetyl-Al, particularly when analyzed according to the EURL-SRM method for fosetyl-Al, imperiling the export of tree nuts to the EU (valued at $2.7 billion in 2014). The results from a multi-instrument, inter-laboratory study demonstrating the limitations of the current EU methodology are presented along with alternative methods of analysis utilizing liquid chromatography (LC-MSMS) and capillary electrophoresis (CE-MSMS).

Five "blinded" ground nut samples, from the International Nut and Dried Fruit Council, were divided between three laboratories for triplicate analysis using the EURL-SRM (QuPPE) extraction method and/or a novel methanolic extraction. Three different LC-MSMS triple quad systems were used for sample analysis (from Agilent, Shimadzu and Waters) and two different types of liquid chromatography (anion exchange and reverse phase columns) were employed for quantitation of phosphonate residues. The effect of phosphonate source and separation technique was examined by analyzing solvent and matrix spikes of phosphonic acid, phosphoric acid and fosetyl-Al.

The inter-laboratory study revealed several deficiencies in the EURL-SRM *"Quick Method for the Analysis of Residues of numerous Highly Polar Pesticides in Foods of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination (QuPPe-Method)"* when used to quantify fosetyl / phosphonate levels in tree nuts, including poor extraction efficiency and low repeatability. Of particular concern is the false positive for phosphonate observed in the presence of high levels of phosphate when analyzing with negative ionization. High levels (≥500 µg/mL) of phosphoric acid were injected while monitoring the MRM transitions for phosphonate. No phosphonate was present in the samples, but the false positive from a level of phosphate regularly found in California tree nuts gave enough of a signal for phosphonate to trigger the EU MRL for fosetyl. For duplicate injections of tree nut extracts analyzed with positive and negative ionization, negative ionization yielded a consistently higher phosphonate concentration. Positive ionization mode has a significantly lower response factor for phosphonate, compared to negative mode (5-10x lower), but has no spectrometric interference from phosphate.

Capillary electrophoresis, coupled to tandem mass spectrometry (CESI-MSMS), has been found to be a promising alternative to LC-MSMS analysis. Preliminary results show that a high degree of electrophoretic resolution can be achieved between phosphonate and phosphate and that retention times will remain stable throughout the lifetime of a capillary (LC chromatographic resolution quickly degrades). Matrix suppression also has been shown to be nearly nonexistent with CESI-MSMS analysis, compared to a ≥60% reduction in signal in LC-MSMS (with great variance across samples).

12:00 – 12:40

Dr. Nicolas Heinzel
Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany

Nicolas Heinzel, Hardy Rolletschek
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany

Method Development with CESI-MS in Plant Science

ABSTRACT: To unravel mechanisms of plant growth and development it is crucial to quantify metabolic intermediates, including their spatial and temporal dynamics. The most widely, analytical approach relies on the use of LC/MS technology. This approach, however, is often limited by sample availability, for example when the plant material is reduced to one small slice of a few micrometer thickness and/or submillimeter area. To overcome this limitation we are developing analytical procedures relying on laser-microdissection and micro-extraction of plant material and subsequent analysis using capillary electrophoresis (CE) coupled to mass spectrometry. Analysis using the instrument CESI8000 requires only minor tissue amounts, because of its ability to handle with minimum sample volume (~5 µl) in a high sensitivity range (nanoliter flow). The aim is to accurately quantify individual metabolic intermediates in microsamples (< 100 µg) and/or even individual cells. The CE/MS approach is also valuable when combined with chemical imaging technologies like magnetic resonance imaging or FTIR microscopy. CE/MS is used to cross-validate metabolite maps generated by these imaging techniques, e.g. to determine the abundance of phytohormones, sugar phosphates or amino acids in distinct regions of plant organs (as specified by previous metabolite mapping). In this presentation we demonstrate the overall work flow, the analytical methods for CE/MS and first results for some amino acids and phytohormones.

Notes:

12:40 – 13:20

Dr. Andreas Schmidt
Ludwig-Maximilians-University of Munich, Germany

CESI-MS in Combination with SWATH Acquisition for the Analysis of Tryptic Digests

ABSTRACT: TBC

Notes:

14:00 – 14:40

Dr. Stephen Lock
SCIEX, United Kingdom

Peptide Quantitation by CESI-MS.

ABSTRACT: Despite recent developments in liquid chromatography column and hardware technology analysis of larger intact peptides, for example Pituitary adenylate cyclase-activating peptide (PACAP), Vasoactive intestinal peptide (VIP) and insulin by liquid chromatography mass spectrometry (LCMS) remains challenging. These larger peptides often exhibit properties which adversely affect their chromatography. In some instances they are polar eluting in the solvent front or overly retained causing peak broadening or problems with carryover and absorption to surfaces. Capillary electrophoresis (CE) is an established and powerful technique for intact protein and peptide analysis, particularly in the characterization of biopharmaceutical drugs such as Insulin and monoclonal antibodies[1]. CESI connects CE to a MS detector and is the integration of CE and ESI into one dynamic process, within the same device (deemed CESI), and it has been shown to provide highly efficient peptide separation and ionization in the ultra-low nanoflow regime (~25 nL/min)[2].

In this presentation we will discuss where CESI has been used in the detection of neuropeptides. We will look at the effect of the capillary coating on the separation and analysis of peptides such as VIP, PACAP, Somatostatin and insulin. We will show the effect of injection type on the analysis and show how this approach can be used to analyse sample volumes from as low as 5 µl or below. Current detection limits depend on the MS detection system but examples of where this technique has been applied to the detection of peptides in matrices including plasma and milk will be discussed.

1. 'Comprehensive Multilevel Characterization of Biologics Using Sheathless Capillary Electrophoresis Hyphenated to Tandem Mass Spectrometry'. R. Gahoual, M. Biacchi, J-M. Busnel, A. Beck, Y-N. François, E. Leize-Wagner, *LCGC*, Volume 13, Issue 4, October 2015
2. 'CESI-MS A new way to analyse for PACAP and VIP'. Stephen Lock, Conference proceedings, 12th International Symposium of VIP-PACAP and Related Peptides, Turkey September 2015.

Notes:

14:40 – 15:20

Professor Alexander R. Ivanov

Northeastern University, Barnett Institute of Chemical and Biological Analysis, Boston, MA

Analysis of Biopharmaceutical Proteins, Protein Complexes and Proteomes Using CESI-MS

ABSTRACT: Proteins, including biopharmaceuticals and building blocks of multimeric protein complexes, typically consist of heterogeneous collections of proteoforms, containing covalent and non-covalent modifications. Accurate knowledge of proteoform profiles is critical for assessing biological functions of proteins and protein assemblies as well as the safety and stability of biopharmaceuticals. Current mainstream methods of analyzing proteins and post-translational modifications (PTMs) require proteolysis or removal of PTM moieties (glycan profiling) that can result in loss of information on correlated abundances of PTMs. Native and top-down mass spectrometry (MS) are emerging fields of MS technology for structural characterization of intact proteins, non-covalent protein-protein and protein-ligand complexes, and macromolecular assemblies under non-denaturing and denaturing conditions. Despite recent progress in top-down and native MS analysis, to date, most MS studies of intact proteins and protein complexes have been based on direct infusion. Both native and top-down MS could greatly benefit from the direct coupling with high-resolution separations. However, the limited choice of separation methods for intact proteins and protein complexes coupled with MS is among the major issues of these MS approaches. Additionally, minimal sample preparation, as used in native and top-down MS, is also desirable to minimize the introduction of artifacts and to study labile PTMs.

Experimental conditions for separation, in-source ion desolvation, fragmentation, and CESI were optimized, and then efficient separation and high sensitivity analysis (e.g., low fmole) were achieved. Additional proteoforms were detected using the native CESI-MS approach in comparison to experiments performed using direct infusion native MS or CESI-MS under denaturing conditions. Protein identities were confirmed using MS/MS. By optimizing sample handling, separation and injection conditions, we were able to resolve glycan isomers of interferon-β1 and determine the correlation in the abundance of certain modifications. We have also been able to profile more than 100 PSA glycoforms under native or denaturing conditions. The performance of the native CESI-MS approach was assessed in the analysis of model mixtures of complex-forming proteins and the *E. coli* ribosomal proteome.

In summary, CESI-MS in combination with advanced MS and bioinformatics techniques has demonstrated the power to provide excellent minimally destructive capabilities for identification and quantitative characterization of proteins, protein complexes, and even proteomes in their native and intact states.

15:20 – 16:10

Professor Jennifer Van Eyk

Advanced Clinical Biosystems Research Institute, Barbra Streisand Women's Heart Center, Heart Institute - Cedars-Sinai Medical Center, Los Angeles CA

Keynote Presentation

A Twist to Increase Specificity in Heart Disease: Analysis of Protein Modifications Using CESI-MS

ABSTRACT: Heart disease continues to be the number one cause of mortality worldwide, which underscores the urgent need for risk stratification to identify individuals at risk of developing heart failure (HF) and/or to monitor HF progression. The two clinical biomarkers primarily used in heart disease, cardiac troponin I (cTnI) and B-type Natriuretic Peptide (BNP), both have disease-induced modifications that, if quantified, will increase disease-state specificity. Thus, an alternative strategy for increasing specificity in monitoring heart disease involves the analysis of disease induced protein modifications as a reflection of an individual's underlying pathophysiology. First, phosphorylation of cTnI alters its ability to regulate cardiac contractility and drives the dysfunction that occurs with advancing HF. Using targeted liquid chromatography coupled with mass spectrometry (LC-MS) we are able to determine the concentration of cTnI, the gold standard for diagnosing a heart attack. We have extended cTnI's diagnostic space by targeting the phosphorylation sites increased with HF as a means to identify those survivors of heart attacks who will proceed to develop lethal HF within two years. The use of capillary electrophoresis coupled to mass spectrometry (CESI-MS) has increased the sensitivity required for detection of cTnI and its phosphorylated forms to allow, for the first time, quantification of these forms in plasma. Second, BNP is a hormone produced by the heart and is the gold standard for assessment of HF and/or hemodynamic dysfunction in a number of other diseases. Currently, this analyte is quantified using clinical chemistry grade ELISA assays directed at either the pro-hormone (NT-BNP) or to BNP (residues 1-32) itself. As BNP is the biologically active form, its measurement should more closely reflect the underlying pathology of HF. Unfortunately, the accurate measurement of BNP is highly controversial owing to its rapid proteolysis upon release into the blood. Accurately measuring these resulting proteoforms, which exist transiently and at low concentrations, is an enormous challenge that is further confounded by plasma collection and storage conditions. Finally, for analytical methods to be adopted for clinical purposes, reproducibility, sensitivity, and accuracy must be twinned to ease of use, turnaround time, and throughput. Using CESI-MS, we have developed assays that provide quantitative information about both the proteolysis rate of BNP in blood, as well as the proteoforms that are produced. Our method involves minimal sample processing that can be completed in less than ten minutes, with intra- and inter-run reproducibility of ≤ 20% Using multi-segment CESI we can produce a profile of BNP cleavage in plasma, including sample processing and analysis, in under one hour.

See how

CESI-MS

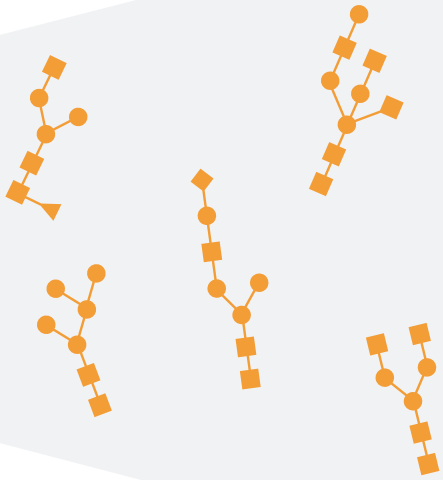
can help you

drive innovations

in biopharma,

proteomics, &

metabolomics.



MISS A SESSION OR WANT TO SEE IT AGAIN?

WATCH RECORDED PRESENTATIONS ON DEMAND

https://sciex.6connex.com/event/eSymposium/CESI/login