2018

Global CESI-MS Symposium

Leiden, The Netherlands | October 10-11, 2018

Leiden University Medical Center (LUMC)

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Hosts and Symposium Chairs:

Professor Manfred Wuhrer
Leiden University Medical Center, The Netherlands

Professor Govert Somsen
VU University, The Netherlands

Session Chairs:

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

Professor Claire Eyers
University of Liverpool, UK

Professor Jennifer Van Eyk
Cedars-Sinai Medical Center, USA

Richard Snell
GSK, UK

Professor Govert Somsen
VU University, The Netherlands

Dr. Spencer Walse
USDA, USA

Professor John Yates III
The Scripps Research Institute, USA

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Day 1 – Proteomics Research

Time	Description
09:50 – 10:50	**Registration**
10:50 – 11:00	**Welcome and Introduction** *Symposium Chair and Your Host, Professor Manfred Wuhrer* *Leiden University Medical Center (LUMC), The Netherlands*
11:00 – 11:30	**CESI-MS: A Complementary Technique to LC-MS for the Identification of Post-translational Protein Modifications** *Professor Herbert Lindner, Innsbruck Medical University, Austria*
11:30 – 12:00	**Key Note Presentation – Characterization of Intentional and Naturally Occurring Modifications in Proteins by CESI-HRMS** *Dr. Elena Dominguez-Vega, Leiden University Medical Center, The Netherlands*
12:00 – 13:00	**Lunch and Poster Session** | *Next Session Chair: Professor Jennifer Van Eyk*
13:00 – 13:30	**Identification of Snake Venom Protein by CESI-MS** *Professor Xin-Xiang Zhang, Peking University, China*
13:30 – 14:00	**Analysis of Intact Prostate Specific Antigen by CESI-MS** *Alan Moran, Leiden University Medical Center, The Netherlands*
14:00 – 14:30	**Key Note Presentation – The Top to Bottom of Protein Phosphorylation Using CESI-MS** *Professor Claire Eyers, University of Liverpool, UK*
14:30 – 15:00	**Coffee Break** | *Next Session Chair: Dr. Guinevere Lageveen-Kammeijer*
15:00 – 15:30	**Corona Isolation Method Matters: CESI-MS-Based Comparison of Corona Compositions Following On-Particle Versus In-Solution or In-Gel Digestion** *Dr. Klaus Faserl, Innsbruck Medical University, Austria*
15:30 – 16:00	**Key Note Presentation – The Future Fight Against Heart Disease: The Need to Observe More with Less** *Professor Jennifer Van Eyk, Cedars-Sinai Medical Center, USA*
16:00 – 16:40	**Open Forum – Panel Discussion on Applications of CESI-MS Proteomics**
16:40 – 16:50	**Concluding Remarks – Day One** *Professor Govert Somsen, VU University, The Netherlands*
18:30 – 22:00	**Conference Excursion** *Join us for a tour of the historic Leiden Astronomical Observatory, followed by a reception at the Castle Oud-Poelgeest.*

11:00 – 11:30

Professor Herbert Lindner

Innsbruck Medical University, Austria

CESI-MS: A Complementary Technique to LC-MS for the Identification of Post-translational Protein Modifications

INTRODUCTION: Post translational modifications (PTMs) are important indicators of change in cells. Two of the top four most abundant PTMs are deamidation and phosphorylation. LC-MS methods struggle to identify and quantify Aspartate and iso-Aspartate isomers (associated with deamidation) as they have the same mass and similar fragmentation patterns which often result in false positives. Location of the phosphorylation site is important in understanding the effect of this modification on the activity of proteins. Phosphorylated peptides are often polar and elute early in traditional LC analyses and positional isomers of phosphory-lated peptides are identical in mass and have very similar fragmentation patterns which makes their iden-tification difficult by LC-MS.

METHODS: For the CESI separation 7.5 nL of sample were injected onto either a positively coated or neutrally coated fused-silica capillary. The separation conditions depended on the capillary coating, but neutrally coated capillaries separations used 30 kV (normal polarity mode), with a pressure gradient of 0–43 min, 0.5 psi; 43–51 min, 2 psi; and 51–60 min, 5 psi. Acetic acid 10% (v/v) was used as the BGE for neutrally coated capillaries. For Nano LC analysis digests were analyzed using an Ultimate 3000 Nano-HPLC system with a homemade frit less fused silica micro-capillary column (75 µm inner diameter x 280 µm outer diameter) packed with 15 cm of 3 µm RP C18 material and using an acetonitrile/water gradient elution at a flow rate of 250 nL/min. Both separations techniques used a Thermo Scientific Orbitrap MS system operated in data-dependent mode to detect the peptides.

PRELIMINARY DATA: This work shows that CESI-MS is able to separate by charge peptides from minute (<20 nL) injection volumes with sample consumption less than 1% of that by LC-MS allowing the analysis of limited samples firstly by CESI-MS and then by LC-MS thereby increasing peptide coverage. Both techniques are orthogonal, but CESI-MS can detect unique PTM sites which were missed when only LC-MS was used. CESI-MS can also provide the separation and detection of mono-phosphorylated peptide isomers, sites of citrullination and other challenging PTMS in a single analysis of biological samples.

NOVEL ASPECT: CESI-MS comparison with Nano-LC for PTM analysis

11:30 – 12:00

Dr. Elena Dominguez-Vega, T.P. Sénard, C. Gstöttner, G.S.M. Lageveen-Kammeijer, D. Falck, S. Nicolardi, M. Wuhrer

Leiden University Medical Center, The Netherlands

KEY NOTE PRESENTATION

Characterization of Intentional and Naturally Occurring Modifications in Proteins by CESI-HRMS

INTRODUCTION: Post-translational modifications (PTMs) of proteins significantly affect their structure and function and are the target of many scientific research. Over last decades, capillary electrophoresis (CE) has demonstrated by far to be an excellent technique to separate intact proteins and their proteoforms. CE has the intrinsic capacity to produce narrow peaks for large macromolecules and the selectivity to separate closely-related protein variants and isoforms. Thanks to recent technological developments in MS-interfacing, CE can be now coupled to mass spectrometers via nano-ESI resulting in high sensitivity. In particular, when coupled with high resolution mass spectrometers (HRMS), such as orbitrap or FTICR, reliable assignment of diverse and/or co-occurring post-translational modifications is attainable.

METHODS: Analyses of proteins were performed using CESI-MS. Sheathless integrated capillary electrophoresis electrospray ionization was carried out on a CESI 8000 instrument (Sciex, Brea, CA) coupled to either an Impact qTOF mass spectrometer or a 12 tesla SolariX XR™ FTICR from Bruker Daltonics equipped with a nano-electrospray source. Protein separations were performed using in-house polyethylenimine (PEI) coated capillaries with a porous tip. Solutions of 1-20% acetic acid containing 0-5% isopropanol or 25 mM ammonium acetate (pH 7.0) were used as background electrolytes.

PRELIMINARY DATA: This lecture shows the power of CESI-HRMS for the assessment of PTMs of intact proteins relevant to pharmaceutical and biomedical sciences. Various CESI-MS approaches, have been developed and applied to study of PTMs which have been intentionally induced (e.g. by glycoconjugation) or that naturally occurs in proteins (e.g glycosylation, deamidation or oxidation). To prevent protein adsorption, we employed dedicated positively-coated capillaries providing efficient protein separations. The flexibility of CESI to be hyphenated with various MS analyzers has been exploited during our research and adapted to the specific needs. First results of the hyphenation of CE with FTICR will be shown. Recent results on the developed CESI-MS systems will be described with special focus on glycoprotein vaccine candidates and biopharmaceuticals. Analysis of Fc portions prepared from human plasma IgG will be shown which permits the allotype-specific study of glycosylation and co-occurring modifications. The proposed approaches provided reliable assignment of a variety of PTMs including deamidated forms.

NOVEL ASPECT: Detailed characterization of new candidate vaccines and Fc portions of IgGs captured from human plasma. Hyphenation of CE with FTICR MS.

13:00 – 13:30

Professor Xin-Xiang Zhang,[1] Ying Liu,[1] Xiao-Hui Zhang,[1] Ying-Lin Zhou,[1] Hong-Xu Chen[2]

1 Peking University, China
2 SCIEX, China

Identification of Snake Venom Protein by CESI-MS

INTRODUCTION: Venomous snakes are widely found almost everywhere in the world, which cause extensive public health problems. Snake venom is a complex mixture mainly consisting of proteins and peptides. The composition of snake venom varies with different genera, species, and even locality. These variations lead to different mechanisms of poisoning, such as neurotoxic, hemotoxic, and cytotoxic pathologies and lead to different anti-venom serums for treatment. In addition, people use different species of snake venom to develop new drugs for treatment of multiple diseases, such as haemostatic disorders, hypertension, thrombosis and cancer. It is of great importance to identify species of snakes from venoms for treatment, medicolegal examination of snakebites and quality control of pharmaceuticals.

METHODS: 10% HAc was used as running buffer for peptides analysis, while 1% FA was used for protein. A voltage of +25 kV and a pressure of 2.0 psi was applied during the separation. ESI was performed in positive ionization mode and detection was operated in MS2 full scan mode. After the separation, the MS spectra were processed by ProteinPilotTM software.

PRELIMINARY DATA: This system achieved high sensitivity (1 μg/mL in 5 μL, Figure 1.a) and high selectivity (none characteristic peptides found).

NOVEL ASPECT: Identification of Snake Venom Protein by CESI-MS

13:30 – 14:00

Alan Moran,[1] E. Dominguez Vega,[1] J. Nouta,[1] T.M. de Reijke,[2] M. Wuhrer,[1] G.S.M. Kammeijer[1]

1 Leiden University Medical Center, The Netherlands
2 University of Amsterdam, Amsterdam, The Netherlands

Analysis of Intact Prostate Specific Antigen by CESI-MS

INTRODUCTION: The analytical platform, capillary electrophoresis electrospray ionization coupled to mass spectrometer (CESI-MS) provides an excellent analysis technique for the in-depth characterization of a protein and its modification variants, such as glycosylation. These variants can influence the two main parameters that allow separation in CE, namely, charge and hydrodynamic volume. This study aimed to evaluate the potential of CESI-MS for the characterization of prostate-specific antigen (PSA), a clinical biomarker of prostate cancer (PCa), on an intact and (glyco)peptide level comparing the information obtained with these two approaches. The intact analysis provides information about different proteoforms while the bottom-up approach allows a deeper insight in the overall glycosylation of specific glycopeptides.

METHODS: Patients suspected of PCa donated a urinary sample prior to digital rectal examination and prostate biopsy. Capturing of the PSA from 20 mL of urine was performed by immuno-purification as described previously 1. A patient pool (5 mL per patient) and a healthy female urine pool were used as positive and negative controls, respectively. Notably, samples were divided into two fractions for intact protein analysis (80%) or digestion with trypsin for glycopeptide analysis (20%). Analysis was performed using a sheathless capillary electrophoresis electrospray ionization mass spectrometer (CESI-MS) on a CESI-8000 instrument (SCIEX) coupled to an Impact quadrupole time-of-flight (QToF)-MS (Bruker) employed with a nano-electrospray source.

PRELIMINARY DATA: The analysis of intact PSA revealed several different PSA glycoforms and proteoforms. Mono-, di-, tri-sialylated complex-type glycans and high-mannose types (Man3 till Man5) were observed, and a diantennary disialylated glycan with a core fucose (H5N4F1S2) was observed as the most abundant N-glycan. In total, 15 glycoforms were identified and in addition, three proteolytic cleavage sites (Asn108/Arg109/Phe110, Lys169/Lys170, and Lys206/Ser207) were found. In the pooled urinary PSA sample, the internal cleavage at a lysine residue was the most prominent form. Unlike commercially-available seminal plasma PSA also non-glycosylated PSA was observed for urine. In addition, variation in the abundancies of proteoforms and glycoforms illustrated the clear differences that are present between urinary PSA and seminal PSA. Though, the significance of this is not yet known, Linton et al. (2003) showed that internally cleaved PSA seems to be more elevated in cases of benign prostate hyperplasia (BPH) in comparison with PCa 2. This suggests that differences in the abundances of certain proteoforms (and glycoforms) could be of use for distinguishing these patient groups. Furthermore, the analysis of urinary PSA glycopeptides provided a broader coverage of N-glycan structures on the single N-linked glycosylation site (Kammeijer et al. 2017). While the bottom-up approach did not allow to look for associations between certain glycopeptides and other PSA modifications, it enabled the separation the isomeric species differing in α2,6- and α2,3-linked sialic acids. Interestingly, α2,3-linked sialylation has been mentioned by Ishikawa et al. (2017), as a potential glycan biomarker of PCa 3. In total 67 N-glycans could be identified, revealing the complementarity between the two analytical approaches as well as the high potential of using

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CESI-MS as an analytical platform for biomarker discovery. Future research should provide information as to whether the observed differences in proteoforms and specifically glycoforms between PCa patient samples may have potential as markers of PCa.

NOVEL ASPECT: Revealing the broad application of CESI-MS by examining the glycoprotein prostate-specific antigen with intact analysis as well as with a bottom-up approach.

REFERENCES.

1. Kammeijer, G.S.M, Nouta, J., de la Rosette, J.J., de Reijke, T.M. and Wuhrer, M., 2018. An In-Depth Glycosylation Assay for Urinary Prostate-Specific Antigen. *Analytical Chemistry*, 90(7), pp.4414-4421.
2. Linton, H.J., Marks, L.S., Millar, L.S., Knott, C.L., Rittenhouse, H.G. and Mikolajczyk, S.D., 2003. Benign prostate-specific antigen (BPSA) in serum is increased in benign prostate disease. *Clinical Chemistry*, 49(2), pp.253-259.
3. Ishikawa, T., Yoneyama, T., Tobisawa, Y., Hatakeyama, S., Kurosawa, T., Nakamura, K., Narita, S., Mitsuzuka, K., Duivenvoorden, W., Pinthus, J.H. and Hashimoto, Y., 2017. An Automated Micro-Total Immunoassay System for Measuring Cancer-Associated α2, 3-linked Sialyl N-Glycan-Carrying Prostate-Specific Antigen May Improve the Accuracy of Prostate Cancer Diagnosis. *International Journal of Molecular Sciences*, 18(2), p.470.

14:00 – 14:30

Professor Claire Eyers

University of Liverpool, UK

KEY NOTE PRESENTATION

The Top to Bottom of Protein Phosphorylation Using CESI-MS

INTRODUCTION: Protein post-translational modifications (PTMs) are critically important in regulating protein structure and function, often in a rapid and reversible manner. Mass spectrometry (MS) has become the technique of choice for analysing PTMs. Whilst 'bottom-up' peptide-based proteomics is routinely applied and offers advantages in terms of ease of analysis and lower limit of detection for characterisation of sites of modification, connectivity between PTMs on a single polypeptide chain is lost. In contrast, 'top-down' proteomics, yields unique and highly valuable information on the connectivity and therefore combinatorial effect of multiple PTMs in the same polypeptide chain.

METHODS: CESI-MS together with a polyethylene imine (PEI) coated capillary was used to analyze samples injected hydrodynamically. Background electrolytes containing methanol and acetic acid were used to separate proteins under reverse polarity conditions due to the positive capillary coating. Proteins were detected using an Orbitrap LUMOS or FUSION MS enabling top down sequencing.

PRELIMINARY DATA: The results showed that CESI-MS was capable of separating intact phosphoproteins based on the level of their phosphorylation. Separating proteins that carry different combinations of modifications to allow multiple PTM sites to be defined using MS is inherently difficult. In this presentation we will be able to demonstrate how capillary electrophoresis (CESI) in combination with high resolution MS can be used to explore PTM space and proteoform heterogeneity.

NOVEL ASPECT: CESI-MS of Intact Phosphoproteins

15:00 – 15:30

Dr. Klaus Faserl, Andrew J. Chetwynd,[2] Iseult Lynch,[3] James A. Thorn,[2] Herbert H. Lindner[1]

1 Innsbruck Medical University, Austria
2 SCIEX, UK
3 University of Birmingham, UK

Corona Isolation Method Matters: CESI-MS-Based Comparison of Corona Compositions Following On-Particle Versus In-Solution or In-Gel Digestion

INTRODUCTION: Nanomaterial protein corona is important in nanomaterial uptake into, and impacts on, cells and organisms, and the consequent need for characterization of the corona has led to a flourishing of methods for isolation and analysis of the constituent proteins. However, despite over 700 corona studies to date very little is understood in terms of which methods provide the most precise and comprehensive characterization of the corona. With the rising importance of modelling of corona formation and its correlation with biological impacts, it is timely to properly characterize and validate the isolation approaches used to determine the protein corona. The current work develops an on-particle tryptic digestion method, comparing peptide solubilization solutions and characterizing the recovery of proteins from the nanoparticle surface.

METHODS: This work compares a variety of in-solution digestions and an in-gel digestion of the protein corona. CESI-MS using a neutrally coated capillary was used to analyze samples injected hydrodynamically. Peptides were detected using a Q-Exactive HF in DDA mode and every sample was injected in triplicate. Data acquired using CESI-MS was also compared to a standard Nano LC-MS approach.

PRELIMINARY DATA: The results showed that the on-particle digest enabled the detection of the most peptides and proteins in the shortest time. However, the range of different proteins classes recovered varied between the different methods. Apolipoproteins were detected at much lower concentrations when a surfactant was used to solubilize peptides, whereas immunoglobulins in general have a high affinity for nanoparticles and thus show a lower recovery using on-particle digestion. These are important factors to consider when designing corona studies and modeling corona formation and impacts, highlighting the significance of a comprehensive validation of nanomaterial corona analysis methods.

NOVEL ASPECT: CESI-MS of Nanoparticle Corona

15:30 – 16:00

Professor Jennifer Van Eyk

Cedars-Sinai Medical Center, USA

KEY NOTE PRESENTATION

The Future Fight Against Heart Disease: The Need to Observe More with Less

INTRODUCTION: The heart's primary task is to contract in order to circulate blood throughout the body. The structural myofilament proteins are responsible for cardiac muscle contraction. The myofilaments dominant the cardiac proteome accounting for over 70% of the myocyte by mass making it a more challenging proteome to process and analyze compared to most of cells types that are typically studied. It is also no surprise that these proteins are regulated by post-translational modifications that effect protein structures, interactions, signaling and which have important implications on cardiac health and disease.

METHODS: Rat myoblast cells (H9c2) were grown under standard tissue culture conditions, DMEM with 10% FBS, penicillin/streptavidin and grown at 37° C in 5% CO_2. Cells were collected, and the soluble fraction and insoluble/myofilament fractions were extracted. Aliquots of both fractions were removed for total protein analysis and the remaining sample was used for phosphopeptide enrichment using TiO_2. Rat myoblast and human embryonic kidney cells were used to test methods to increase proteome coverage. All samples were analyzed using either Easy-Spray nano-LC column on a Orbitrap Fusion Lumos or CESI using a neural capillary on a Q Exactive Plus. Peptide fragmentation spectra were generated using HCD.

PRELIMINARY DATA: CESI-MS was used to analyze the proteome and phosphoproteome from myocytes and compare peptide identifications to data generated on reverse phase LC-MS (Orbitrap Fusion Lumos) to define a subset of peptides previously undetectable or with enhanced detection by CESI-MS (Q Exactive Plus). The whole cellular proteome was analyzed in two protein fractions, the soluble and myofilament, to test the increase in proteome depth and minimize sample requirements. Our goal is to minimize sample needs while maximizing proteome coverage to facilitate high throughput analysis of low number of cells and tissue amounts to address the area of heart disease.

NOVEL ASPECT: Myocyte proteome and phosphoproteome by CESI-MS

Day 2 – BioPharma, Glycomics and Small Molecule Analysis

Time	Description
09:00 – 09:30	**Registration**
09:30 – 09:40	**Welcome and Introduction** *Symposium Chair and Your Host, Professor Govert Somsen* *VU University, The Netherlands*
09:40 – 10:10	**High-Order Structural Characterization of Monoclonal Antibodies by Combination of Intact, Middle-Up and Bottom-Up Techniques Using CESI-MS** *Dr. Yannis Francois, University of Strasbourg, France*
10:10 – 10:40	**Fluorescence Detection of APTS Labeled Carbohydrates at the Taylor Cone in CESI-MS** *Dr. Andras Guttman, University of Debrecen, Hungary*
10:40 – 11:10	**Key Note Presentation – Is the Signature Peptide for Quantification a Thing of the Past? Using CESI-MS to Quantify mAbs with a Pseudo Intact or Intact Approach** *Richard Snell, GSK, UK*
11:10 – 11:30	**Coffee Break** | *Next Session Chair: Professor Herbert Lindner*
11:30 – 12:00	**Extracting More Chemical Information Using Less Sample Material by CESI-MS** *Dr. Rawi Ramautar, Leiden Academic Center for Drug Research, The Netherlands*
12:00 – 12:30	**Key Note Presentation – MEKC-MS: From Supposedly Incompatible to Low-ng/L Sensitivity** *Professor Govert Somsen, VU University, The Netherlands*
12:30 – 13:30	**Lunch and Poster Session** | *Next Session Chair: Dr. Steve Lock*
13:30 – 14:00	**A Comparison of the Challenges in the Quantification of Highly Polar Compounds with Agricultural Significance Using CESI-MSMS and LC-MSMS** *Dr. Wiley Hall, Dried Fruit Association of California, USA*
14:00 – 14:30	**The Detection of Pesticide Metabolite TFA by CESI-MS** *Dr. Sven Stuke, Bayer, Germany*
14:30 – 15:00	**Key Note Presentation – Environmental and Agricultural Applications of CESI-MS** *Dr. Spencer Walse, USDA, USA*
15:00 – 15:20	**Coffee Break** | *Next Session Chair: Professor Manfred Wuhrer*
15:20 – 16:00	**Key Note Presentation – Comprehensive Analysis of HIV Glycosylation by Capillary Electrophoresis Separation and Ultraviolet Photodissociation** *Professor John Yates III, The Scripps Research Institute, USA*
16:00 – 16:10	**Concluding Remarks – Day Two**
16:10 – 16:30	**Adjourn**

09:40 – 10:10

Dr. Yannis Francois,[1] Jérémie Giorgetti,[1] Nassur Saïd,[1] Rabah Gahoual,[2] Alain Beck,[3] Emmanuelle Leize-Wagner[1]

1 University of Strasbourg, France
2 University of Paris, France
3 Pierre Fabre Immunology Center, France

High-Order Structural Characterization of Monoclonal Antibodies by Combination of Intact, Middle-Up and Bottom-Up Techniques Using CESI-MS

INTRODUCTION: MAbs are highly complex glycoproteins potentially displaying many naturally-occurring molecular micro-heterogeneities combined with imperfect processing, physico-chemical and enzymatic changes during their production and long term conservation. There is a continuous need for analytical methods improvement to be able to provide a fast and accurate characterization. We propose the development of complementary approaches implementing CESI-MS to characterize the different aspects defining the structure of several mAbs. In the first level CE-MS enabled accurate mass. Middle-up analysis allowed to study independently Fc/2 and F(ab')2 subunits. Finally, from a single injection complete primary structure of mAbs could be characterized using a CE-ESI-MS/MS methodology.

METHODS: Samples were analyzed using a CESI 8000 instrument (SCIEX, Brea, CA), coupled to a 5600+ TripleTOF (SCIEX, Darmstadt, Germany) mass spectrometer. For intact and middle-up analysis, mAbs was buffer exchanged with 20 mM ammonium acetate buffer, pH 7.0 after or not IdeS digestion. For peptide mapping, samples were digested by trypsin using an in-solution digestion protocol. Samples were finally diluted to a final concentration in protein of 2.2 µM using ammonium acetate 50 mM, pH 4.0. Separations were performed in neutral, positive or bare-fused silica capillaries (length, 95 cm; i.d., 30 µm) while background electrolyte was acetic acid 10% to 30 %.

PRELIMINARY DATA: The first step of a mAb structural characterization is the mass measurement of the intact molecule. CE-ESI-MS method enables a profile spectrum of the whole antibody giving access to the confirmation of elemental composition and the characterization of major glycoforms. Within a single run of few minutes and consuming less than 3µg mAbs sample, measured masses with good accuracy were in good agreement with already published values. To investigate deeper the characterization of mAbs, middle-up analysis using IdeS enzymatic digestion has been performed following the same experimental protocol used for intact mAbs analysis. Structural information of the expected $F_{c/2}$ subunits and F(ab')2 subunits have been obtained. Finally, we confirmed the use of sheathless CE-ESI-MS/MS for the bottom-up analysis of mAbs to enhance the level of characterization. Using a single injection of 200 fmol, we were able to characterize the primary structure of those mAbs in a robust manner. We managed to perform the complete amino acid sequence characterization. Simultaneously, using the same set of data, glycosylations and post-translational modification (PTM) were also characterized. Concerning glycosylation, 14 different N-glycans could be characterized and quantified for each mAbs. The same analysis also enabled the precise characterization of all PTMs known as "hot-spots" used to monitor product stability, such as deamidation of asparagine, oxidation of methionine and isomerization of aspartic acid. Validation of the CE-ESI-MS method in terms of robustness and reproducibility was demonstrated for ten different mAbs produced in different cell lines.

NOVEL ASPECT: Intact and middle-up level characterization using CE-MS. Complete mAbs primary structure characterization (sequence, glycosylations, PTM). Relative quantitation of glycosylation profiles.

10:10 – 10:40

Dr. Andras Guttman, Mate Szarka, Marton Szigeti

University of Debrecen, Hungary

Fluorescence Detection of APTS Labeled Carbohydrates at the Taylor Cone in CESI-MS

INTRODUCTION: Analysis of N-linked carbohydrates of glycoproteins are routinely done by CE at the released glycan level by LIF detection. Glycan level analysis necessitates endoglycosidase mediated release of the carbohydrate moieties and charged fluorophore tagging to accommodate electric field mediated separation. For the analysis part, albeit CE-LIF fulfills the industry requirement of high-resolution analysis, simultaneous MS detection offers additional information for fully comprehensive structural elucidation especially for complex samples such as human serum. Integration of CE and electrospray ionization (ESI) into a single dynamic process, i.e., within the same device (CESI) offers the capability of performing CE separation at ultra-low nanoliter flow rates, resulting in reduced ion suppression and improved sensitivity for MS detection.

METHODS: N-Glycans were released by PNGase F digestion and labeled with aminopyrenetrisulfonate (APTS) for both CE-LIF and CE-MS analysis using the Fast Glycan Sample Preparation and Analysis kit (SCIEX, Brea, CA). All capillary electrophoresis analyses were performed on a CESI 8000 Plus system using simultaneous laser induced fluorescence (LIF) and mass spectrometry (QTRAP® 6500+) detection (all from SCIEX). CESI-MS separations were achieved using an OptiMS bare fused-silica capillary cartridge by the application of -30 kV and low pressure (2 - 5 psi). Acetic acid and low pH ammonium ammonium acetate buffers were used as background electrolytes (BGE).

PRELIMINARY DATA: The released and APTS labeled glycans were analyzed in negative ESI mode, scanning from 250 - 2000 m/z with scan speed of 250 ms (MS). The use of CE-MS in negative ionization mode revealed that reversed CE polarity offered high separation efficiency. The combination of EOF and pressurization resulted in an optimum flow rate of ~20 nL/min, maximizing ionization efficiency and minimizing ion suppression. Simultaneous LIF and MS detection offered full quantification option. Using the optimized methods readily enabled detailed analysis of the N-glycosylation of therapeutic antibodies and human serum samples at the released N-glycan levels.

NOVEL ASPECT: Fluorescence detection of N-glycans in the Taylor cone of the electrosrpay with picomolar sensitivity.

10:40 – 11:10

Richard Snell

GSK, UK

KEY NOTE PRESENTATION

Is the Signature Peptide for Quantification a Thing of the Past? Using CESI-MS to Quantify mAbs with a Pseudo Intact or Intact Approach

INTRODUCTION: TOF-MS can provide more than just small molecule quantitation to the modern bioanalytical lab, in a time when molecules are becoming more diverse, and the needs of project are moving beyond what standard approaches can supply, can HR-MS provide a solution? TOF-MS is a common tool in peptide mapping and characterisation, but coupled with capillary electrophoresis (CE), HR-MS offers a robust and sensitive solution for quantitative bioanalysis of monoclonal antibodies (mAbs) Using a pseudo intact approach rather than using signature.

METHODS: CESI-MS together with a neutrally coated capillary was used to analyse samples injected electrokinetically. Background electrolytes containing acetic acid combined with a stacking injection approach, which used ammonium acetate, were used to separate proteins under a split pressure normal phase separation. Proteins were detected using TOF-MS.

PRELIMINARY DATA: Using a pseudo intact approach rather than using signature peptides CESI-MS has been shown to provide representative quantitative data, as well as potentially providing information about post translational modifications (PTMs) in tox samples, that may affect activity.

By adjusting the ammonium acetate stacking concentration and injection conditions CESI run times have been reduced to less than 18 minutes. Separation of mAb fragments has been maintaining by using a twostep pressure approach in the separation. Migration times and peak areas have been shown to be very reproducible aided using mineral oil which eliminated evaporation effects on the sample.

NOVEL ASPECT: CESI-MS of Intact Protein Quantitation

11:30 – 12:00

Dr. Rawi Ramautar, Wei Zhang, Thomas Hanekemeier

Leiden Academic Center for Drug Research, The Netherlands

Extracting More Chemical Information Using Less Sample Material by CESI-MS

INTRODUCTION: The analytical toolbox used in metabolomics is still facing difficulties for the analysis of highly polar and charged metabolites in biological samples. Especially the selective and sensitive profiling of acidic metabolites, including sugar phosphates and nucleotides, remains a highly challenging analytical task in metabolomics. Even more when considering the analysis of such compounds in limited amounts of sample material. As a result, a significant number of crucial biomedical questions cannot be addressed by the current metabolomics approach. Here, the performance of CESI-MS for the global profiling of charged metabolites in biological samples is demonstrated. The applicability is especially shown for the analysis of these challenging compounds in microscale cell cultures.

METHODS: Sheathless interfacing of a CESI8000 instrument (Sciex) and a high end TOF-MS instrument was achieved through a capillary of which the outlet was etched with hydrofluoric acid, providing an outer terminus porous to the electric transport of small ions. The separation capillary with this porous tip was placed in a stainless steel ESI needle filled with static conductive liquid. ESI was performed in both positive and negative ionization mode (1.2 kV) and 10% acetic acid (pH 2.2) was used as background-electrolyte.

PRELIMINARY DATA: The use of CESI-MS with an injection volume of only 20 nanoliter resulted in low nanomolar detection limits for a broad array of polar metabolites, including sugar phosphates, organic acids and nucleotides. Structural isomers as well as isobaric metabolites could be selectively analyzed without using any derivatization. A unique feature of the proposed approach is that it allows profiling of both acidic and basic metabolites using a single capillary/buffer combination by only switching the MS detection and separation voltage polarity. The utility for analyzing the polar metabolome was investigated for limited amounts of HepG2 cells, which was used as a model for microscale cellular systems.

NOVEL ASPECT: Low-flow CE-MS approach for global profiling of polar and charged metabolites in volume-limited samples using a single capillary/buffer combination

12:00 – 12:30

Professor Govert Somsen, David Moreno-González,[1] Rob Haselberg,[2] Laura Gámiz-Gracia,[1] Ana M. García-Campana,[1] Gerhardus J. de Jong[3]

1 Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Spain
2 Division of BioAnalytical Chemistry, AIMMS, Vrije Universiteit Amsterdam, The Netherlands
3 Biomolecular Analysis, Utrecht University, The Netherlands

KEY NOTE PRESENTATION

MEKC-MS: From Supposedly Incompatible to Low-ng/L Sensitivity

INTRODUCTION: Micellar electrokinetic chromatography (MEKC) is a powerful mode of CE, which enables electrophoretic separation of neutral components by analyte partitioning between an aqueous and a pseudo-stationary phase (PSP) of charged micelles. The on-line coupling of MEKC and mass spectrometry (MS) is problematic and hampered by incompatibility problems leading to reduced separation performance and poor limits of detection (LODs).

METHODS: We evaluated the potential of porous-tip sheathless interfacing (CESI) for MEKC-MS with the objective to attain strongly improved LODs. In order to achieve full compatibility with positive ESI-MS detection, perfluorooctanoic acid was used as surfactant creating a volatile PSP for MEKC separations. Carbamate pesticides (CRBs) were selected as representative model compounds being neutral toxic pollutants potentially present at trace levels in environmental water samples. MEKC-MS/MS system parameters were optimized with focus on interfacing conditions and MS settings in order to reach high analyte signal-to-noise ratios (S/Ns).

PRELIMINARY DATA: A background electrolyte of 75 mM perfluorooctanoic acid adjusted to pH 9.0 with ammonium hydroxide allowed efficient separation of 15 CRBs and appeared fully compatible with ESI-MS. For CRBs the LODs ranged from 0.2 to 3.9 ng/L (13 nL injected), representing an improvement of more than 300-fold when compared with conventional sheath-liquid interfacing. Good linearity ($R2 > 0.99$) and satisfactory reproducibility were obtained for all CRBs with interday RSD values for peak area and migration time of 4.0-11.3% and below 1.5%, respectively. Analysis of spiked mineral water showed that the new MEKC-MS/MS method allows selective and quantitative determination of CRB concentrations below the maximum residue limit of 100 ng/L without the need for sample pre-concentration.

NOVEL ASPECT: A new fully compatible MECK-MS/MS method is presented, providing efficient separation and selective detection of neutral compounds with unsurpassed sensitivity.

13:30 – 14:00

Dr. Wiley Hall

Dried Fruit Association of California, USA

A Comparison of the Challenges in the Quantification of Highly Polar Compounds with Agricultural Significance Using CESI-MSMS and LC-MSMS

INTRODUCTION: Quantitating residues of highly polar pesticides is of great interest 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, and phosphonic / phosphoric acids), and the large amounts of these compounds that are used each year. Analysis with LC-MSMS using stationary phases including: graphitized carbon, ion exchange, HILIC, and mixed phase as well as IC-MSMS, have been reported, but these methods can have disadvantages such as matrix suppression, poor retention time stability, and a limited number of analytes that can be analyzed within a single method. There is no polar counterpart to the reversed phase multi-residue screens capable of testing for hundreds of compounds.

METHODS: CESI-MSMS, has been found to be a promising alternative to LC-MSMS analysis. Treenut matrices, fortified with highly polar pesticides, such as glyphosate, glufosinate, paraquat and diquat, were extracted using a modified version of the QuPPe and analyzed with CESI-MSMS using a variety of different background electrodes as well as bare and neutral coated capillaries. CESI-MSMS was also used to elucidate the fate of several different forms of phosphorous oxides (phosphite and phosphate) from different agricultural products (fertilizers, fungicides, etc.) in the environment along with how different form of phosphorous might affect LC-MSMS analyses.

PRELIMINARY DATA: CESI-MSMS has been highly effective for the analysis of charged pesticides including glyphosate, AMPA, glufosinate, fosetyl-Al, phosphonic and phosphoric acids, ethephon, paraquat, diquat, mepiquat, and chlormequat in tree nut matrices, all within a single run (vs 3 runs on different columns with LC-MSMS). Additionally, due to the low flow rate though the CE capillary, matrix suppression is greatly reduced, with linear response achieved over 2 orders of magnitude.

When examining different commercial products containing POx (as acids, P2O5, or salts with a wide variety of counter ions) several different peaks corresponding to the fragments of H3PO4, H3PO3 and their polymers were observed.

NOVEL ASPECT: CESI MS/MS used for the analysis of highly polar pesticides.

14:00 – 14:30

Dr. Sven Stuke,[1] P. Bemboom,[1] H. Wirkner,[1] Whitney Smith[2]

1 Bayer, Germany
2 SCIEX, Germany

The Detection of Pesticide Metabolite TFA by CESI-MS

INTRODUCTION: In the past years, the technology for trace residue analysis of plant protection compounds in plant and animal matrices, soil, and water has changed stepwise to meet changing regulatory demands. While, from the 70s to the 90s of the last century, generally the active compounds and only a few major metabolites had to be determined in a typical "residue definition", further metabolites have since come into the authorities' focus, step by step, within the framework of product safety assessments and of the enforcement of residues in dietary matrices and in the environment.

While many active substances were formerly determined via GC-based detection techniques, the introduction of LC-MS(MS) technology in the 90s and the official acceptance of this coupling technique by official bodies at the end of the 90s led to a major change for residue analytical laboratories all over the world. Most of the medium to non-polar actives as well as most of the more polar metabolites are accessible via this technique, and today, LC-MS/MS is the "workhorse" in many residue analytical laboratories in the industry responsible for analyzing registration-related field studies, but also in official enforcement labs.

With the demand to analyze further breakdown products, more and more polar compounds—or even (permanently) charged target compounds—now come into the focus of the registration authorities. This again brings the LC-based techniques to their limits, and makes approaches such as HILI-chromatography MS/MS or ion chromatography necessary—with all of the related uncertainties and problems in matrix samples.

We will present a new CE-MS/MS-based approach; a GLP-validated residue analytical method for charged compounds (in this case, TFA and DFA) in matrix samples from field residue trials, using a Sciex CESI8000 capillary electrophoresis coupled to a Sciex API6500 triple quadrupole mass spectrometer (CE-MS/MS).

METHODS: CE-MS/MS

PRELIMINARY DATA:

TFA: Recoveries determined acc. EU/OECD Guidelines		
Wheat Green Material	@ 0.1 mg/kg: 101, 101, 101, 105, 103% @ 0.5 mg/kg: 94, 95, 90, 96, 89%	
Linearity TFA	0.5 – 100 µg/L; IS 25 µg/L r= 0.99996 (1/X)	
Incurred Samples; 3 times extr. + Analyzed	Carrot Root	0.397 mg/kg; 0.387 mg/kg; 0.382 mg/kg
	Lettuce Head	0.399 mg/kg; 0.392 mg/kg; 0.404 mg/kg

NOVEL ASPECT: Connection between CE (CESI8000) and MS/MS (API6500+) for (permanently) charged analytical targets.

14:30 – 15:00

Dr. Spencer Walse, Erik Rangel

USDA, USA

KEY NOTE PRESENTATION

Environmental and Agricultural Applications of CESI-MS

INTRODUCTION: This work addresses the application of capillary electrophoresis with mass spectrometry (CESI-MS/MS) to a diverse variety of agricultural, ecological, environmental applications. Commercialized by SCIEX, CESI-MS/MS has revolutionized the spectrometric analysis of highly polar natural products and environmental toxicants, including protic organics, formerly prone to matrix suppression and chromatographic complications associated with reversed phase and ion exchange techniques. Of particular interest was the CESI-MS/MS-quantification of amines that are subject to N-nitrosation, a structural modification that is not chromatographically distinct, at least with conventional approaches, and often is spectrometrically masked, such as during electron-impact ionization.

METHODS: Morpholine and diphenylamine were were fumigated with nitric oxide. N-nitrosation was confirmed with CESI-MS/MS analysis with a CESI-MS-Neutral OptiMS cartridge. Free solution mobility enabled compartmentalization of analytes separated into zones that are then further separated by their electrophoretic mobility and size when an electric field is applied. Structural assignment was based on agreement with synthetic standards. Migration time and mass spectrometry was used for chemical verification and the integral of peak area of [M + H]+, referenced relative to linear least squares analysis of a 5-point concentration – detector response curve, was used to determine the concentration.

PRELIMINARY DATA: Morpholine (MOR) and diphenylaminewere (DPA) were fumigated with nitric oxide. Product analyses showed that N-nitrosodiphenylamine (NDPA) as well as 2-nitrodiphenylamine (2NDPA) and N-nitrosomorpholine (NMOR) formed from DPA and MOR, respectively. Features of the electrophoretic and mass spectrometric resolution will be detailed, as will the rational for method optimization. Discussion will focus on the use of CESI-MS/MS to detect these and other cancer-causing N-nitrosamines in agriculture, personal care products, and disinfection technologies.

NOVEL ASPECT: CESI-MS/MS quantification of amines, formerly prone to matrix suppression and chromatographic complications associated with reversed phase and ion exchange techniques.

15:20 – 16:00

Professor John Yates III, Jolene Diedrich

The Scripps Research Institute, USA

KEY NOTE PRESENTATION

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.

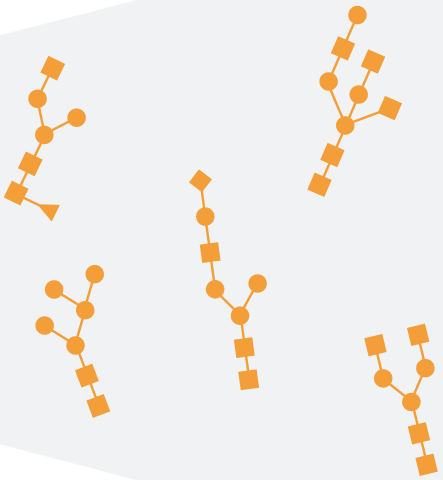
METHODS: We demonstrate glycopeptide analysis on a hybrid quadrupole mass filter/linear ion trap/Orbitrap mass spectrometer, which has been modified to allow ultraviolet photodissociation (UVPD) ion fragmentation. CESI (Capillary Electrophoresis with Electrospray Ionization) and reverse phase UPLC are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

See how

CESI-MS



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