CESI-MS - A Sensitive and Versatile Approach for Metabolomics & Polar Molecules

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INTRODUCTION

The analysis of small polar molecules present an analytical challenge, particularly metabolites, nucleotides/nucleosides, polar pesticide and glycosaminoglycans (GAGs). Further, these classes of analytes present many structural isomers which often can't be differentiated by MS or MS/MS alone, making a separation crucial.

Unfortunately, the liquid chromatography (LC) separations of small, highly charged molecules often proves difficult; some hydrophilic analytes are poorly retained by reversed phase (RP)-LC necessitating ion-paring agents, while column-to-column reproducibility can sometimes be more challenging with hydrophilic interaction liquid chromatography (HILIC). Furthermore, the interface between ion chromatography (IC) and MS has inherently limited compatibility.

In contrast, capillary zone electrophoresis (CZE) is well suited for the analysis of these challenging charged and polar molecules. CESI-MS integrates CE and electrospray ionization (ESI) into a single device, effortlessly combining the benefits of a high-resolution separation with the increased MS sensitivities that result from the ultra-low flow rates.[1]

Many of these benefits have already been seen for the post-translational modification analysis on biological[2] and therapeutic[3] proteins, hydrophobic peptide quantitation,[3] and top-down proteomic analysis.[5,6] In this work, we demonstrate the versatility of CESI-MS for the analysis of these challenging polar metabolites, polar pesticides/herbicides and GAGs.

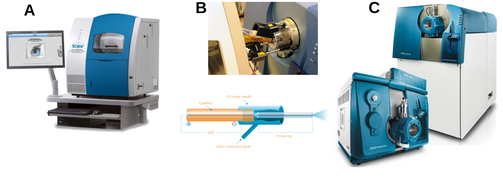

Figure 1. A. CESI 8000 Plus, B. CESI-MS interface, the etched porous capillary tip allows electrospray generation without dilution of the sample by a make-up liquid. C. TripleTOF® 6600 and QTRAP® 6500

MATERIALS AND METHODS

Sample Preparation: Metabolite and polar pesticide/herbicide standards were acquired from Sigma-Aldrich. Heparin disaccharides were acquired from Galen Laboratory Supplies. Samples were diluted in either 10% background electrolyte (BGE) or a 100 mM ammonium acetate pH 4 which acted as the leading electrolyte (LE).

CESI Conditions: All experiments were carried out on a SCIEX CESI 8000 Plus. A bare fused-silica OptiMS cartridge was used for all analyses. Acetic acid (3-10%) or ammonium bicarbonate (pH 9 – 10) was used as the BGE. In negative ion mode 10% isopropanol was added to the BGE. Approximately, 1 - 50 nL of sample was injected hydrodynamically.

MS Conditions: A SCIEX TripleTOF® (5600 or 6600) or a QTRAP® 6500+ system equipped with a NanoSpray® III source and CESI adapter was used for all analysis. Data acquisition was controlled by Analyst® software. Positive or negative ESI was performed. For TOF MS, 75 - 1000 m/z was scanned and confirmed with MS/MS. An MRM strategy was employed for QTRAP® systems.

Data Analysis: PeakView® and MultiQuant™ software's were utilized for data analysis.

RESULTS

Metabolite Analysis

The study of metabolites is critical in many fields as it can provide key insight towards understanding drug toxicities, drug interactions and the mechanisms of actions of therapeutics and biomarkers of disease states. Unfortunately, many metabolites are small and highly polar and thus not suited to RPLC-MS. To address this challenge chemical derivatization, ion pairing agents and HILIC are routinely employed, but each approach has unique disadvantages. CESI-MS is a powerful analytical tool uniquely suited towards the analysis of small, polar, highly charged analytes.

Anionic metabolites

The disregulation of metabolites across the central carbon metabolism (CCM) (consisting of glycolysis, gluconeogenesis, pentose phosphate pathway, and tricarboxylic acid (TCA cycle) is often indicative of a diseased state, particularly cancer. These challenging metabolites include isobaric sugar phosphates, nucleotides, phosphocarboxylic and free carboxylic acids.

CESI-MS is powerful for the targeted analysis of CCM metabolites, particularly the small organic acids of the TCA cycle and the isobaric phosphorylated sugars of the pentose and glycolysis pathways (Figure 2).[7,8]

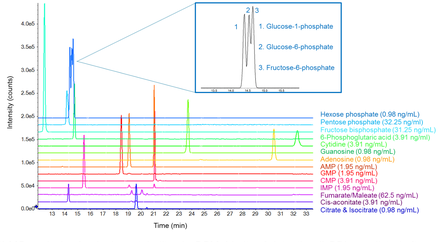

Figure 2. CESI-MS analysis of anionic metabolites in CCM. Limits of detection (LOD)s are listed after each labeled metabolite.

Cationic metabolites

Chimeric antigen-receptor (CAR) T-cells are a promising targeted cancer therapy. In CAR T-cell therapy, indoleamine 2,3-dioxygenase (IDO) modulates the T-cell response and can be monitored to assess the effectiveness of the tumor therapy.

As IDO catabolizes tryptophan, the metabolites of the tryptophan pathway could serve as biomarkers for IDO activity in CAR T-cell therapy. , which makes is necessary to develop a highly sensitive method to analyze these challenging metabolites.

The ultra-low flow rate of CESI-MS increases ionization efficiency and hence facilitates highly sensitive analyses. Here, CESI-MS was evaluated for the analyses of potential biomarkers for IDO (Figure 3 & Table 1).

Table 1. LODs of tryptophan pathway metabolites

Metabolite	LOD (nM)
Nicotinamide	0.56
Serotonin	0.19
3-hydroxy-kynurenine	0.80
L-kynurenine	0.39
Tryptophan	0.11
3-hydroxy anthranilic acid	1.45
Kynurenic acid	0.05
Xanthurenic acid	0.04
Quinolinic acid	0.07

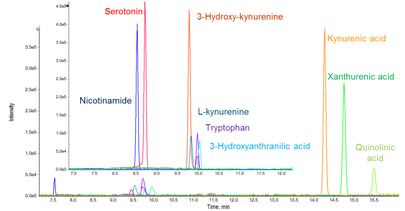

Figure 3. CESI separation of metabolites in the tryptophan pathway

Polar Pesticides & Herbicide Analysis

Concerns about the safety of glyphosate-based herbicides mandate their analysis in foods, especially fruits and nuts. Current IC-MS methods have significant limitations, including ion suppression, retention time instability and difficulty to distinguish alternative herbicides (such as fosetyl aluminum) and degradation products. Additionally, both false positive IDs and inaccurate quantitation of the degradation products (phosphate and phosphonate) are possible with current IC-MS methods.

As CESI is a charged based separation technique it is ideally suited for separations and quantitation of small polar molecules. The CESI technology drastically simplifies the integration of a charged based separation technique with MS. In this example, ions as simple as phosphate and phosphonate have been separated and detected amongst other related pesticides, herbicides that are of common use and their degradation products (Figure 4).[9]

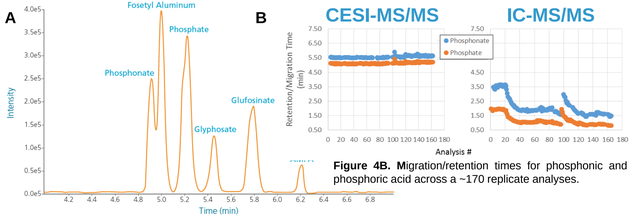

Figure 4A. Separation of polar pesticides

Figure 4B. Migration/retention times for phosphonic and phosphoric acid across a ~170 replicate analyses.

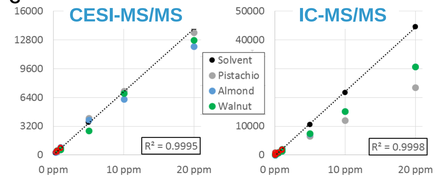

Figure 4C. Identical spikes of phosphonic acid into 0.1% formic acid (solvent) and almond, walnut, and pistachio extracts analyzed by CESI-MS/MS and IC-MS/MS on the TripleTOF® 5600. Black lines represent the linear curve fit for blank solvent calibration curves. Matrix suppression was observed by LC-MS, but not CESI-MS, indicated by curved and linear calibration trends, respectively. Markers outlined in red were samples diluted 5x with 0.1% formic acid.

Glycosaminoglycan Analysis

Heparin is a naturally occurring glycosaminoglycan (GAG). This anticoagulant is employed as a therapeutic for deep vein thrombosis, pulmonary embolism, arterial thromboembolism, heart attacks, and unstable angina. Heparin proves extremely difficult to analyze due to the high negative charge and sequence heterogeneity. The complexity of heparin therapeutics necessitate digestion to smaller oligosaccharides to facilitate the characterization process.

CESI-MS can provide an excellent alternative to HILIC and RP-LC without the need for ion pairing agents. The highly efficient separation can resolve isomers (Figure 5A). Next, the CESI assay is highly sensitive and robust (Figure 5B). Finally, the ability to achieve negative ionization ESI facilitates clean spectra (Figure 5C).

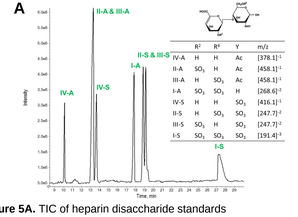

Figure 5A. TIC of heparin disaccharide standards

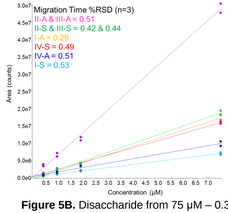

Figure 5B. Disaccharide from 75 µM – 0.36 µM

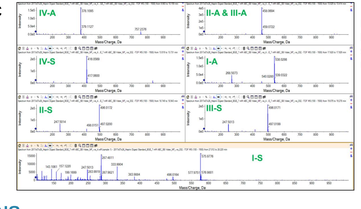

Figure 5C. MS spectra of heparin disaccharide standards in negative ESI mode

CONCLUSIONS

CESI-MS has been demonstrated to be a versatile separation and ionization technique that provides unique separation selectivity's that is highly beneficial for the analysis of challenging small, charged, polar molecules. The highly efficient charged based separation technique was able to resolve isomeric phosphorylated sugars and disaccharides. CESI seamlessly interfaced with MS to provide a highly sensitive and robust analysis of anionic and cation metabolites, polar pesticides/herbicides and GAGs.

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TRADEMARKS/LICENSING

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