From small to very large: orthogonal, sensitive polar molecule analysis by CESI-MS

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ABSTRACT

We present a collection of analyses using CESI-MS that demonstrate the unique capabilities of a low nL/min CE-based separation coupled with mass spectrometry. Notably, all analyses are performed using very similar CE and ESI conditions with simple acetate-based buffers as background electrolytes (BGEs). MS methods are adjusted appropriately based on the net charge (cationic or anionic) and size (~100 Da – 150 kDa) of the molecules. For such we have evaluated a varied collection of real-world analytes, primarily biomolecules, to demonstrate the capabilities of CESI-MS. On the small end of the size scale, ions as simple as phosphate and phosphonate have been separated and detected among other herbicides and their degradation products in common use. Similar molecules in structure, such as isobaric phosphorylated sugars, along with other anionic metabolites can also be resolved and identified in negative ESI mode by a CE-based separation. Larger oligosaccharides, when labeled with an anionic dye like aminopyrene-trisulfonic acid (APTS), enable released glycan analysis from proteins of therapeutic and clinical nature. Moving higher in the mass range, peptides, intact proteins, and native protein complexes can all be separated to identify protein sequence, post-translational modifications, and binding partners. A notable intact protein, monoclonal antibodies (mAb), can be separated to identify charge variants that contribute to stability and efficacy. Collectively these examples illustrate the simplicity of buffer systems that allow for the analysis of a wide range of highly-relevant polar molecules by CESI-MS.

INTRODUCTION

Analysis of polar molecules can present challenges by traditional LC-MS methods that can be addressed by the aqueous nature of capillary zone electrophoresis (CZE)-based separations. The integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into one process (termed CESI) provides the capabilities to improve the sensitivity, speed, and comprehensiveness of polar biomolecule analysis.[1] Many of these benefits have already been realized for post-translational modification analysis on biological[2] and therapeutic[3] proteins, hydrophobic peptide quantitation,[3] and top-down proteomic analysis.[5, 6]

MATERIALS AND METHODS

Sample Preparation:
Samples were diluted ~10-fold in either 10% BGE or a leading electrolyte (LE) and loaded into nanoVials (P/N 5043467) for ~15 - 60 nL pressure injections. Glycan samples were released from proteins with PNGase and labeled with APTS prior to dilution for analysis.

CESI Conditions:
CESI experiments were carried out with a SCIEX CESI 8000 Plus system equipped with a temperature controlled auto sampler and a power supply with the ability to deliver up to 30 kV in normal and reverse polarity. Samples were injected hydrodynamically using 5 psi for 1 - 60 sec corresponding to ~ 1 - 50 nL injection volumes. CESI separations were achieved using either an OptiMS bare fused-silica capillary cartridge (P/N B07367), neutral capillary cartridge (P/N B07368), or cationic polymer-coated capillary[7] with application of -30 to 30 kV and pressure (0 - 5 psi). Acetic acid and ammonium acetate (pH 2 – 4.5) with up to 10% isopropanol was used as the BGE.

MS/MS Conditions:
A SCIEX TripleTOF® 5600 or 6600 system with a NanoSpray® III source and CESI adapter were used, controlled by Analyst® TF 1.7 Software. Positive or negative ESI was performed at ~1500 V scanning from 75 - 4500 m/z with 50 - 250 ms TOF MS, product ion MS/MS, and information dependent analysis (IDA) MS/MS scan accumulation time ranges.

Data Analysis:
High resolution MS and MS/MS spectra were analyzed using SCIEX PeakView®, MultiQuant™, and BioPharmaView™ softwares.

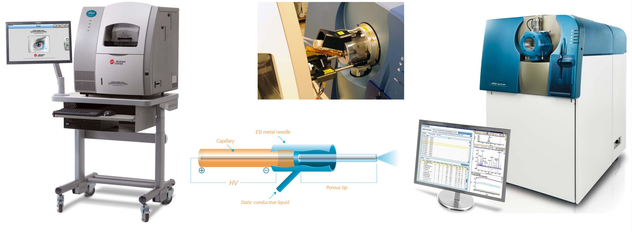


Figure 1. CESI 8000 Plus High Performance Separation-ESI Module coupled to a TripleTOF® 6600 System. The core of the CESI-MS technology is an etched porous capillary tip that allows for electrospray ionization without dilution of the BGE and sample by a make-up liquid. Instead a conductive liquid is used to apply the ESI voltage through the porous tip.

RESULTS

POLAR PESTICIDE AND HERBICIDE ANALYSIS. Polar pesticides and herbicides can present analytical challenges using traditional LC-MS methods. Capillary electrophoresis is ideally suited for separations and quantitation of polar ions. In this case, the separation of phosphonic and phosphoric acids, among other related polar molecules (Figure 2A), facilitates the reproducible differentiation (Figure 2B) and quantification (Figure 2C) of fosetyl aluminum degradation product phosphonate. Notably this can be performed in the presence of much higher natural abundances of the very similar phosphate ions and has reduced ion suppression from matrix.[8]

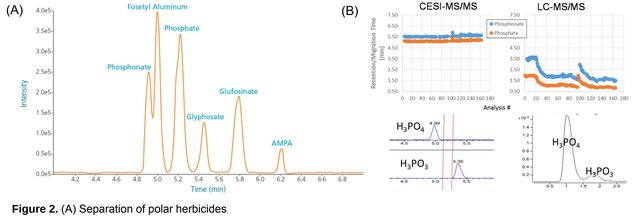
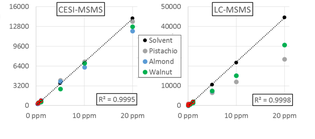


Figure 2. (A) Separation of polar herbicides glyphosate and fosetyl aluminum; their degradation products, phosphate, phosphonate, and aminomethylphosphonic acid (AMPA); and pesticide glufosinate.

(B) Comparison of migration and retention times for phosphonic and phosphoric acid across a ~170 run analysis, with sample electropherograms (CESI-MS/MS) and chromatograms (LC-MS/MS). Baseline resolution is achieved in the CESI-MS/MS analysis.



(C) Identical spikes of phosphonic acid into 0.1% formic acid (solvent) and almond, walnut, and pistachio extracts analyzed by CESI-MS/MS and LC-MS/MS. 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.

ANIONIC METABOLITE ANALYSIS. Similar to polar pesticides, LC-MS methods have short-comings in terms of separation resolution and sensitivity for the analysis of anionic metabolites. In particular, the resolution of isobaric metabolite isomers can be particularly challenging. CESI-MS has benefits in terms of separation selectivity and sensitivity for the analysis of anionic metabolites[9] with data shown specifically for phosphorylated sugar isomers (Figure 3).

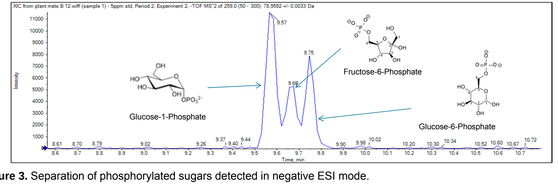


Figure 3. Separation of phosphorylated sugars detected in negative ESI mode.

RELEASED GLYCAN ANALYSIS. Glycans have roles in cellular interactions, protein folding, and protein stability in general and disease biology. Glycans are released and analyzed from individual proteins, such as therapeutics, and also from mixtures as complex as serum. The combination of a CE separation and MS/MS detection facilitates identification and quantification of glycans from limited sample amounts.

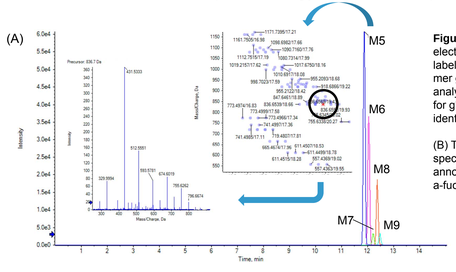


Figure 4. (A) Extracted ion electropherogram of released, APTS-labeled mannose 5, 6, 7, 8, and 9-mer glycans from RNAse B. IDA analysis generated multiple spectra for glycan peaks to confirm identifications.

(B) The combined MS and MS/MS spectra of APTS-glycans can be annotated to identify fucosylated and a-fucosylated glycan structures.

INTACT PROTEIN ANALYSIS. Charge heterogeneity analysis of monoclonal antibodies is a crucial biopharmaceutical characterization. Traditional methods using cation exchange (CEX) chromatographic fractionation with intact mass, peptide mapping, and capillary isoelectric focusing is labor intensive and can take days to weeks to complete. CESI-MS analysis of a commercial, intact therapeutic mAb yielded a charge heterogeneity-based separation in ~ 15 min (Figure 6A). Deconvoluted masses (Figure 6B) were consistent with expected mass shifts, within the error of the mass detection, from a prior characterization by CEX[10].

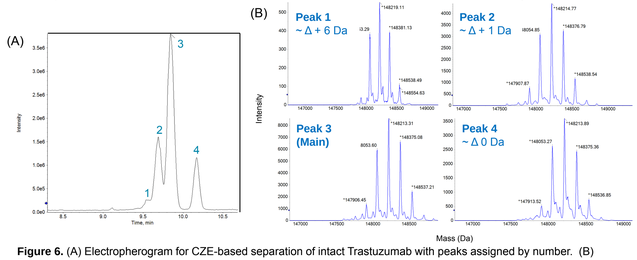


Figure 6. (A) Electropherogram for CZE-based separation of intact Trastuzumab with peaks assigned by number. (B) Deconvoluted MS spectra for each electrophoretic peak.

CONCLUSIONS

CESI is a versatile separation and ionization methodology that provides unique benefits for the analysis of charged, polar molecules in terms of separation selectivity and sensitivity. Very similar CZE conditions have been applied to the separations of a wide range of molecules using the appropriate MS detection conditions. From nL injection volumes, analyses were performed from nanograms to picograms of sample.

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