Increasing Throughput with Multi-Segment-Injection-Capillary Electrospray Ionization-Mass Spectrometry (MSI-CESI-MS)

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Introduction

Any process or technique that provides substantial improvement in throughput has the potential to be a disruptive analytical event. MSI with CESI-MS has this potential to increase throughput by at least an order of magnitude.

CZE and CESI-MS make this possible because of inherent higher peak capacities and better resolution over conventional instrumental techniques such as GC-MS or LC-MS.

MSI is a multiplexing technique in which multiple samples are injected onto the capillary prior to applying the separation voltage. Each sample is injected followed by a spacer solution which provides a differentiating gap between samples in the injection process. It is important to design the injection process and MS detection scheme to allow easy identification of analytes within complex sample output.

The samples are dissolved in an electrolyte which provides peak focusing via transient isotachophoresis (t-ITP), resulting in increased signal to noise. The electrolyte is selected which has a high mobility cation such as NH_4^+ which facilitates the t-ITP event (1).

Background

Injection of multiple samples of pharmaceuticals using capillary zone electrophoresis was reported some years ago by Veuthey et. al. (2).

Britz-McKibbin and co-workers reported the use of multiple sample injections in the analysis of metabolomics analytes (3) and applied the technique in a number of studies. They further referred to the methods as MSI-CE-MS (Multiple Segment Capillary Electrophoresis Mass Spectrometry).

We report here an extension to that work resulting in a high throughput protocol which takes advantage of the low flow, high peak capacity, resolution and sensitivity of CESI-MS technology (Figure 1).

As an example which demonstrates the effectiveness of this technique, methamphetamine and its metabolite, amphetamine were spiked into volunteer urine samples, extracted and reconstituted for analysis.

Multi-Segment Injection (MSI) techniques, were applied using CESI-MS and the protocol was evaluated using an 8 or 6 sample MSI injection approach. Regression analysis was applied to the data in a proof of principle evaluation to assess the potential of this application.

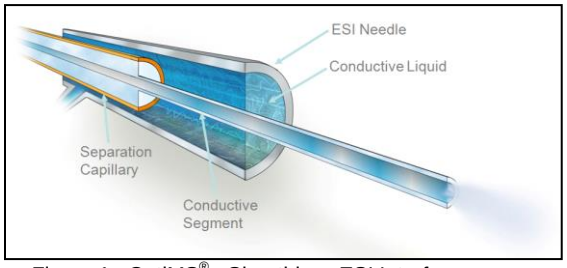

Figure 1: OptiMS® - Sheathless ESI Interface.

Materials and Methods

Chemicals: All chemicals were Reagent Grade and were purchased on-line from VWR International.

Drug and Metabolite Standards: Amphetamine, methamphetamine and their D11,deuterated internal standards, all at 1 mg/mL were purchased from Cerilliant Corporation, Round Rock, TX, USA. These standard solutions in methanol were diluted and spiked into volunteer pooled urine samples. Standard solutions for mass spectrometry and extractions were prepared at 1 ng/µL in 5 to 50 mM Ammonium Formate (pH 2.85).

Urine Calibration Standards: Urine samples were prepared at 2000 ng/mL. These spiked urine sample were diluted with blank urine to prepare calibrators from 1 to 2000 ng/mL. The samples were kept at ~4°C until the time of analysis. Spiked urine samples and blanks were prepared by liquid-liquid extraction after the addition of internal standards at 50 ng/mL.

Instrument Conditions and Extraction Protocol: Tables 1 and 2 outline CESI-MS parameters and Multiple Reaction Monitoring (MRM) transitions that were used for confirmation and quantitative processing. Samples were prepared using the extraction procedure in Figure 2.

OptiMS Capillary Interface	90 cm bare fused silica capillary, 150 µm OD, 30 µm ID with conductive emitter tip
CE Instrument	CESI 8000
MS Instrument	Waters Xevo with MassLynx 4.1 Software
ESI Voltage	1.25 kV
Sample Introduction	Multiple Segment Injections, 2 psi for 5 s with BGE 60 s spacer
Capillary Conditioning	Initial conditioning with MeOH, water, 0.1N NaOH, 0.1N HCl, water and BGE
Background Electrolyte (BGE)	10% Acetic Acid
Separation	30 kV, 333 V/cm, 3.7 µamp
Temperatures	Capillary 25°C Samples 10°C

Table 1: CESI 8000 with Opti-MS® Conditions

Compound	Parent (m/z)	Daughter (m/z)	Dwell	Cone (V)	Collision (V)
Amphetamine MRM1	136.11	91.03	0.05	15	15
Amphetamine MRM2	136.11	119.09	0.05	15	8
D11-Amphetamine	147.17	98.06	0.05	20	17
Methamphetamine MRM1	150.06	91.07	0.05	20	15
Methamphetamine MRM2	150.06	119.09	0.05	20	10
D11-Methamphetamine	161.21	97.05	0.05	25	18

Table 2: CESI 8000 with Opti-MS® Conditions

To 1 mL of urine (or serum, plasma oral fluid):

1. Add 50 µL of Mixed D11 Internal Standards to 1 mL of urine followed by 0.2 mL of conc. NH_4OH and vortex.
2. Add 5 mL of 1-chlorobutane and shake for 10 min.
3. Centrifuge at 0°C for 10 min. at 3000 rpm.
4. Evaporate at 40°C under N_2 for 10 min. to remove any NH_4OH, then add 10 µL of 1% HCl in MeOH. Vortex and continue to evaporate with N_2.
5. Add 200 µL of 5 mM BGE to each tube and vortex.
6. Transfer to a 200 µL Microfuge tube (Beckman Coulter)..
7. Pressure inject the sample for 10 seconds at 5 psi.

Figure 2: Liquid-Liquid Extraction Protocol for Bio-fluids

Results

Spiked urine samples for Meth and Amp using D11, deuterated Internal Standards (IS) were prepared and analyzed using a liquid-liquid extraction protocol (Figure 2).

Firstly, calibrators and blank extracts were injected using an eight or six sample MSI technique composed of two sequential runs. Both runs included a blank and a blank containing deuterated internal standards which showed no significant carryover. The total analysis time was approx. 80 minutes for both runs (~ 5 minutes per sample). See Figure 3.

Regression analysis on ten point calibrations in triplicate over three orders of magnitude, were linear with R^2>0.995 for both amphetamine and methamphetamine over the range of 1 to 2000 ng/mL of urine (Figure 4).

MSI-CESI-MS analysis of samples were reconstituted in 200 µL of 25 mM ammonium acetate pH 4.4 and injected using 2 psi pressure injections for 5 s. This corresponds to 1.7 nL injected or ~8.5 fg injected from the 1 ng/mL calibrator. LOD/LOQ was the low calibrator, 1 ng/mL for both amphetamine and methamphetamine.

Conclusions

MSI-CESI-MS techniques show the potential to increase sample throughput using CESI-MS by an order of magnitude, therefore decreasing analysis time to less than 5 minutes per sample.

Method development is straightforward using common electrolytes combined with t-ITP. This approach provides the required sensitivity to detect and quantitate small drug molecules and their metabolites at sub-therapeutic levels, less than 2 ng/mL of bio-fluids such as urine.

This approach will be of particular value for routine quantifications of multiple components in applied Toxicology and Metabolomics.

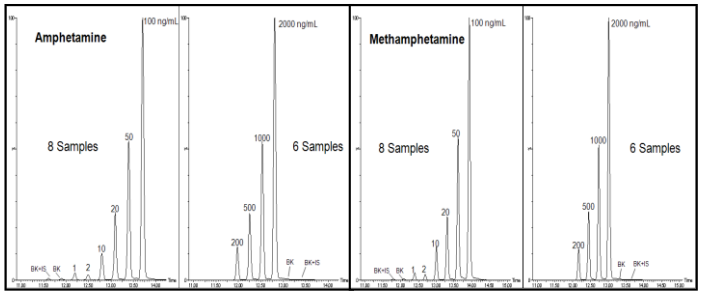

Figure 3: MSI Calibrations for Amp/Meth 8 and 6 Sample Runs

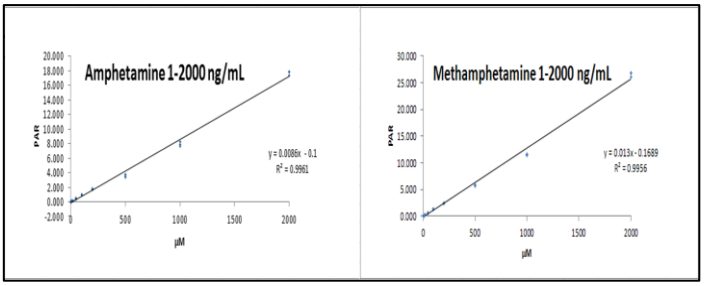

Figure 4: Regression Analysis for Amphetamine and Methamphetamine

References

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