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

John C. Hudson

SCIEX, Regina, SK, Canada

Introduction

Any process or technique that provides substantial improvement in throughput has the potential to be a disruptive analytical event. Multi-segment-injection-capillary electrospray ionization-mass spectrometry (MSI-CESI-MS) has the potential to increase throughput by at least an order of magnitude.

Capillary zone electrophoresis (CZE) and CESI-MS make this possible because of inherent higher peak capacities and better resolution over conventional instrumental techniques such as GC-MS and 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 selected electrolyte has a high mobility cation, such as NH4+, 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 coworkers 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 injection capillary electrophoresis mass spectrometry).

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

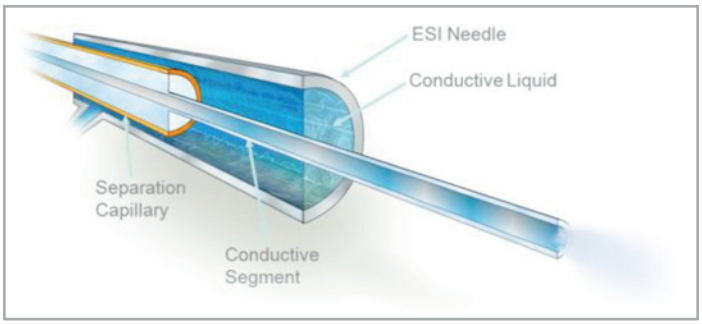

Figure 1. Figure 1. The OptiMS cartridge from SCIEX provides an ultra-low flow ESI interface.

To demonstrate the effectiveness of this technique, methamphetamine and its metabolite, amphetamine, were spiked into volunteer urine samples, and were extracted and reconstituted for analysis.

The multi-segment-injection (MSI) technique was 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.

Materials and methods

Chemicals: All chemicals were reagent grade and were purchased online 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). 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).

Capillary interface	OptiMS cartridge with 90 cm bare fused-silica capillary, 150 µm OD, 30 µm ID with conductive emitter tip
CE instrument	CESI 8000 Plus High Performance Separation-ESI Module
MS instrument 1	SCIEX Triple TOF® 6600 LC-MS/MS System with Analyst® Software 1.7
MS instrument 2	Waters Xevo with MassLynx Software 4.1
ESI voltage	1.25 kV
Sample introduction	Hydrodynamic 5 psi for 10 s
Capillary conditioning	Initial conditioning with MeOH, water, 0.1 N NaOH, water and BGE
Background electrolyte (BGE)	25 mM ammonium formate (pH 2.85)
Separation	25 kV, 277 v/cm, 2.3 µamp
Temperatures	Capillary 25 °C; Samples 10 °C

Table 1. CESI 8000 Plus System with OptiMS parameters.

Compound Name	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	91.03	0.05	15	15
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 Plus System with OptiMS conditions.

To 1 ml of urine (or serum, plasma oral fluid)
1. Add 50 µl of mixed 011 Internal Standards to 1 ml of urine followed by 0.2 ml of cone. 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_40H, 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.

Urine calibration standards: Urine samples were prepared at 2000 ng/mL. These spiked urine samples 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: Table 1 and Table 2 outline the 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.

Important:

- A separation current above 5 µA might cause permanent damage to the separation capillary.
- Generally, please do not apply >2000V to generate electrospray as it may result in capillary damage.

Results

Spiked urine samples for methamphetamine and amphetamine using D11 deuterated internal standards (IS) were prepared and analyzed using a liquid-liquid extraction protocol (Figure 2).

First, calibrators and blank extracts were injected using an 8 or 6 sample MSI technique composed of 2 sequential runs. Both runs included a blank and a blank containing deuterated internal standards, which showed no significant carryover. The total analysis time was approximately 80 min for both runs (~5 min per sample). See Figure 3.

Regression analysis on 10 point calibrations in triplicate over 3 orders of magnitude were linear, with R2>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.

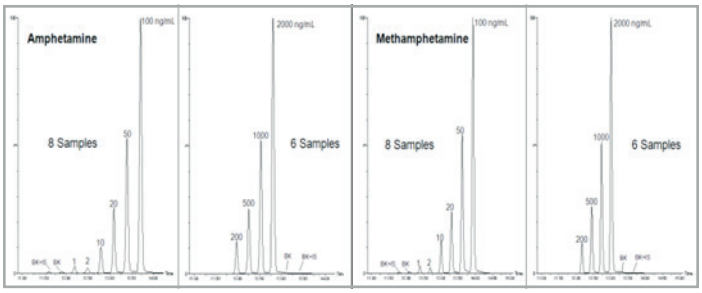

Figure 3. MSI calibrations for amphetamine/methamphetamine 8 and 6 sample runs.

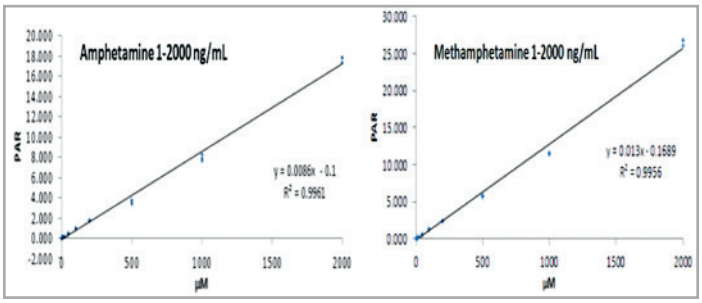

Figure 4. Regression analysis for amphetamine and methamphetamine.

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 min per sample.

Method development is straightforward using common electrolytes combined with t-ITP. This approach provides the required sensitivity to detect and quantify 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.

References

1. Busnel, J.-M. et al. High Capacity Capillary Electrophoresis-Electrospray Ionization Mass Spectrometry: Coupling a Porous Sheathless Interface with Transient-Isotachophoresis. *Anal. Chem.* 2010, 82, 9476–9483.

2. Geiser, L.; Rudaz, S.; Veuthey, J.-L. Decreasing analysis time in capillary electrophoresis: Validation and comparison of quantitative performance in several approaches. *Electrophoresis* 2005, 26, 2293–2302.

3. Kuehnbaum, N.L.; Kormendi, A.; Britz-McKibbin, P. Multisegment Injection-Capillary Electrophoresis-Mass Spectrometry: A High-Throughput Platform for Metabolomics with High Data Fidelity. *Anal. Chem.* 2013, 85, 10664–10669.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701, USA
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