Chiral Analysis of Methamphetamine and its Metabolite, Amphetamine, in Urine by CESI-MS

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Introduction

Chiral separation of drug enantiomers is essential in order to show that the active enantiomer is, in fact, present in forensic specimens. This avoids legal arguments and simplifies challenges to analytical findings. Chiral analysis of methamphetamine (meth) “street” samples yields information on the clandestine lab synthetic route.[1] Chiral analysis is also of great importance in pharma and drug discovery for the detection of chiral impurities and for quantitative determinations.

In the past, chiral analysis has been done using a combination of processes, starting with the drug confirmation by hyphenated mass spectrometry (CE-MS, GC-MS or LC-MS). This was followed by separation of the enantiomers and impurities of the drug by a specific chiral separation technique, such as chiral capillary electrophoresis or chiral chromatography.

Direct connection of chiral separation technology with mass spectrometry can be problematic. The use of chiral GC and LC columns alone or with mass spectrometry provides, at best, marginal separation capability. Furthermore, the addition of neutral or highly sulfated cyclodextrin additives in chromatographic and electro-driven separation modes can cause contamination and ion suppression in the electrospray process.

In 2005, Rudaz and Veuthey[2] showed that adequate chiral separations and identification of enantiomers could be done using a sheath-liquid CE-MS technique. Their partial filling technique (PFT), under countercurrent conditions, employed highly sulfated cyclodextrin (HSCD) additives to a simple background electrolyte (BGE) to separate the enantiomers of amphetamine (amp) derivatives (see Figure 1).

In this work, a low-flow capillary electrophoresis electrospray interface for mass spectrometry (CESI-MS) was used with the partial filling technique, as illustrated in Figure 1, to generate the chiral separation and produce the quantitative data.

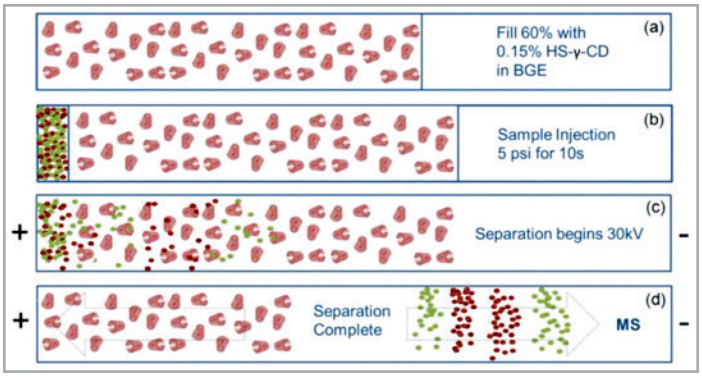

Figure 1. PFT and counterion flow: (a) rinse with BGE followed by 25 psi/60 s injection to partially fill the capillary; (b) inject sample (~8.5 nL) with 5 psi for 10 s; (c) voltage separation at 30 kV and (d) separation complete.

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 TripleTOF® 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 M NaOH, water and BGE
Background electrolyte (BGE)	25 mM ammonium formate pH 2.85
Separation	25 kV, 277 V/cm, 2.3 µA
Temperatures	Capillary 25 °C; samples 10 °C

Figure 2. CESI 8000 with Opti-MS ® Conditions

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.

To 1 ml of urine (or serum, plasma or 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_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 s at 5 psi.

Figure 3. Liquid-liquid extraction protocol for bio-fluids

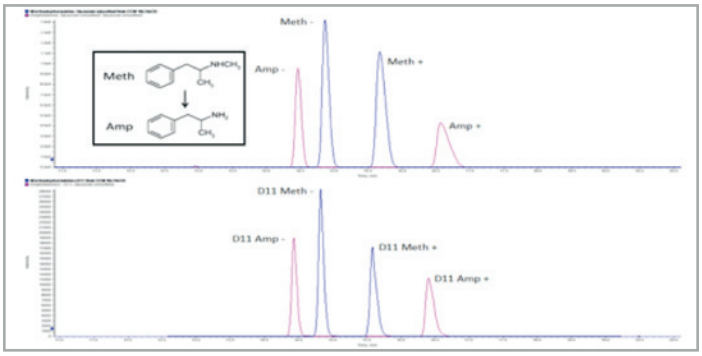

Figure 4. Chiral separation of amp±, meth±, D11-amp± and D11-meth± with a SCIEX TripleTOF® 6600 System and Analyst® Software 1.7.

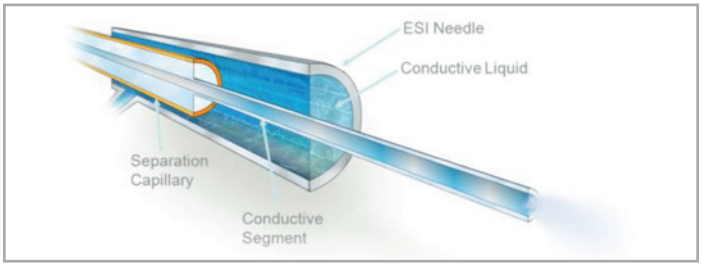

Figure 5. The OptiMS cartridge from SCIEX provides a sheathless ESI interface.

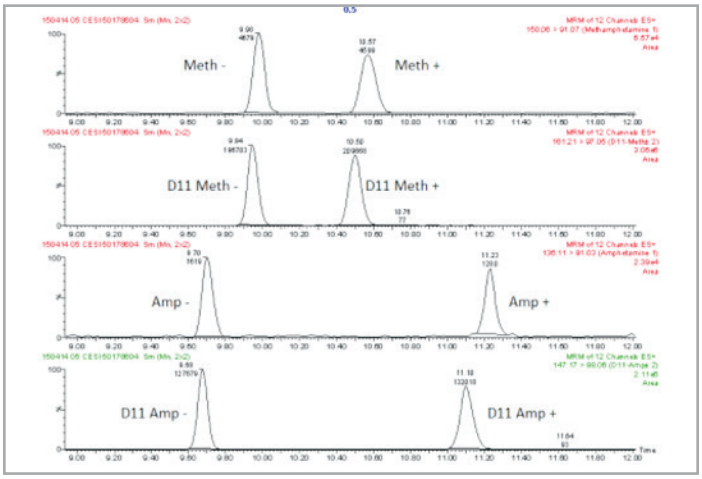

Figure 6. MRM analysis (~20 fg Injected).

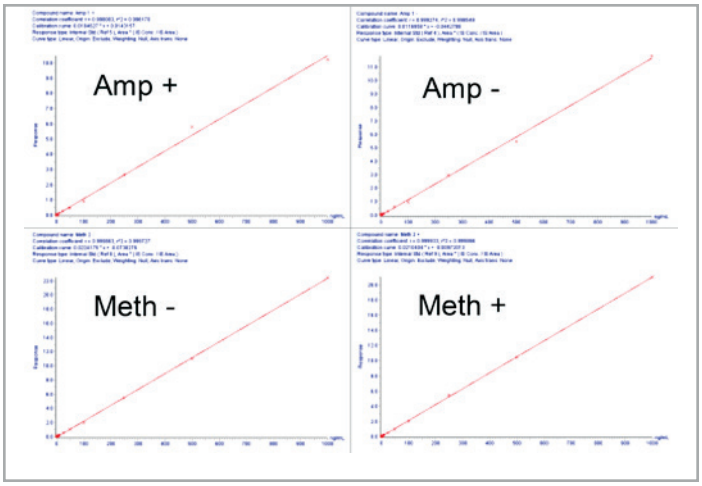

Figure 7. Linear regression analysis for amp and meth enantiomers.

Materials and methods

Chemicals: All chemicals were reagent grade and were purchased online from VWR Int. Highly sulfated-gamma-cyclodextrin (HS-γ-CD), 20% solution in water, was obtained from Beckman Coulter Inc. (Brea, CA).

Drug and metabolite standards: Meth±, amp± 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).

Urine calibration standards: Urine samples were prepared at 2000 ng/mL. These spiked urine samples were diluted with blank urine to prepare calibrators from 0.5 to 1000 ng/mL per enantiomer. 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 of each deuterated enantiomer.

Instrument conditions and extraction protocol: Figure 2 and Figure 3 outline CESI-MS parameters and the liquid-liquid extraction process.

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 3).

The partial filling technique (Figure 1), in which the capillary is 40% filled with BGE containing 0.15% HS-γ-CD, was used to affect the chiral separation of meth and amp (Figure 4).

The CESI 8000 Plus System with the OptiMS cartridge (Figure 5) was used to interface CE and MS, providing the required sensitivity on injections of only 8.5 nL of the extract reconstituted in 200 µL (~20 fg injected).

Multiple reaction monitoring (MRM) was used for the quantitative processing (meth: 150.2 → 119.1, amp: 136.2 → 119.2, D11-amp 147.2 → 98.1, D11-meth 161.2 → 97.1).

The chiral CESI-MS separation for the 0.5 ng/mL spiked urine extract is shown in Figure 6. LOD/LOQ was the low calibrator, 0.5 ng/mL of each enantiomer.

For each enantiomer, 10 point calibrations in triplicate over 3 orders of magnitude were linear with R2 >0.995 for both meth and amp from 0.5 to 1000 ng/mL of urine (Figure 7).

Conclusions

A partial filling technique was adapted to a low-flow capillary electrophoresis electrospray interface for mass spectrometry (CESI-MS).

Chiral separation and confirmation of the enantiomers of methamphetamine and its metabolite, amphetamine, in a single run, were demonstrated as proof of principle at the sensitivity that forensic toxicologists require in even the most challenging case work.

References

1. Iwata, U.T.; Inoue, H.; Kuwayama, K.; Kanamori, T.; Tsujikawa, K.; Miyaguchi, H.; Kishi, T. Forensic application of chiral separation of amphetamine-type stimulants to impurity analysis of seized methamphetamine by capillary electrophoresis. *Forensic Science International* 2006, 161, 92–96.
2. Rudaz, S.; Geiser, L.; Souverain, S.; Prat, J.; Veuthey, J.-L. Rapid stereoselective separations of amphetamine derivatives with highly sulfated cyclodextrin. *Electrophoresis* 2005, 26, 3910–3920.

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