Analysis of Methamphetamine and Its Metabolite, Amphetamine by Chiral CESI-MS

John C. Hudson, Beckman Coulter, Inc.
Brea, CA, USA

Introduction

Chiral separation of drug enantiomers is essential in order to show that the active enantiomer is indeed 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 mass spectrometer.

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 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.

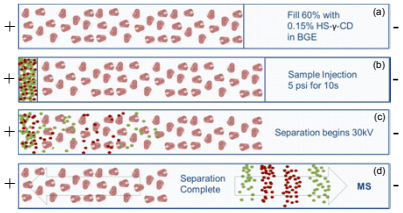

Figure 1: PFT and Counterion Flow, (a) rinse with BGE followed by 25 psi/80 s injection to fill 60% of capillary, (b) inject sample with 5 psi for 10 s, (c) voltage separation at 30 kV and (d) separation complete.

Material and Methods

Chemicals:
All chemicals were Reagent Grade and were purchased on-line from VWR Int. HS-γ-CD, 20% solution in water, was obtained from Beckman Coulter Inc., Brea, CA, USA.

Drug and Metabolite Standards:
Meth and Amp standards were purchased from Cerilliant Corporation, Round Rock, TX, USA. Methoxamine Internal Standard (IS) was obtained from Sigma-Aldrich. Stock solutions prepared at a concentration of 1 mg/mL in methanol and were further diluted for spiking 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 from a volunteer spiked at 50 ng/mL. The spiked urine sample was diluted with blank urine to two other concentrations, 5 and 0.5 ng/mL, for preparation of a three point HS-γ-CD calibrations. The samples were kept frozen until the time of analysis. Spiked urine samples and blanks were prepared by liquid-liquid extraction.

CESI 8000 with Opti-MS® Conditions

OptiMS* Capillary Interface	90 cm bare fused silica prototype 150 µm OD, 30 µm ID with conductive emitter tip
Separation	30kV, 333 V/cm, 2.5 µamps
Temperatures	Capillary 25°C Sample Storage 10°C
Background Electrolyte (BGE)	25 mM Ammonium Formate, pH 2.85
Sample Introduction 25	Hydrodynamic 5 psi for 10 s
CE Instrument	CESI 8000* Prototype
MS Instrument	Waters Xevo TQ with MassLynx 4.1
Conductive Liquid	0.7% Formic Acid
ESI Voltage	1.25 kV
Capillary Conditioning	The capillaries were initially conditioned with MeOH, water, 1N NaOH, water and BGE.

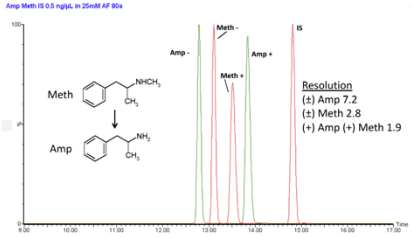

Figure 2: Chiral Separation of Meth, Amp and Methoxamine IS

Biological Fluid Extraction Protocol

To 1 mL of urine, whole blood or serum:

1. Add 10 ng of IS 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 0-4°C for 10 min. at 3000 rpm.
4. Evaporate carefully just to dryness with N_2 or in a SpeedVac®.
5. Add 100 µL of 5 mM BGE to tube, vortex, heat to dissolve extract.
6. Transfer to a 200 µL Microfuge® tube.
7. Centrifuge at 14,000 rpm for 20 min.
8. Pressure inject the sample for 10 seconds at 5 psi.

Figure 3: Liquid-Liquid Extraction Protocol for Biofluids

Results

1. The Partial Filling Technique (Figure 1), in which the capillary is 60% filled with BGE containing 0.15% HS-γ-CD, was used to affect the chiral separation of Meth and Amp (Figure 2).

2. Spiked urine samples for Meth and Amp using Methoxamine as the Internal Standard (IS) were prepared and analyzed using a liquid-liquid extraction protocol (Figure 3).

3. The CESI 8000 with Opti-MS (Figure 4) was used to interface CE and MS, providing the required sensitivity on injections of only 7 nL of extract dissolved in 100 µL.

4. Multiple Reaction Monitoring (MRM) was used for the quantitative processing (Meth: 150.2 → 119.1, Amp : 136.2 → 119.2, Methoxamine IS: 212.4 → 194.4).

5. The Chiral CESI-MS separation for the 0.5 ng/mL spiked urine extract is shown in Figure 5.

6. Three point calibrations for each enantiomer were linear with R^2 >0.99 for both Meth and Amp from 0.5 to 50 ng/mL of urine (Figure 6).

OptiMS® - Sheathless ESI Interface

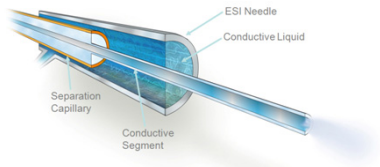

Figure 4: OptiMS® - Sheathless ESI Interface Schematic

Acknowledgements

Facilities: Dr. R. Raina, University of Regina, Regina, SK, Canada

Advice and Review: Staff at Beckman Coulter Inc., Brea, CA, USA.

Amp & Meth Spiked Urine at 0.5 ng/mL

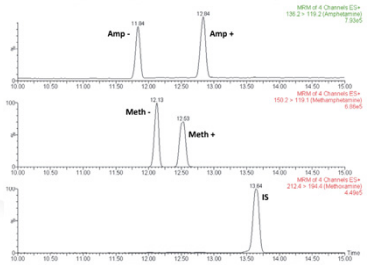

Figure 5: Chiral Separation – MRM Analysis

Linear Regression Analysis

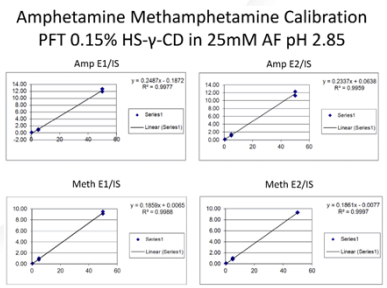

Figure 6: Linear Regression Analysis for Amp and Meth Enantiomers

Conclusions

A Partial Filling Technique (PFT) was adapted to a low flow Capillary Electrophoresis Electrospray Interface for Mass Spectrometry (Chiral CESI-MS).

Chiral separation and confirmation of the enantiomers of methamphetamine and its metabolite, amphetamine, in a single run, were demonstrated at levels of detection (ng/mL of urine) which 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. and T. Kishi "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. and Jean-Luc Veuthey "Rapid stereoselective separations of amphetamine derivatives with highly sulfated cyclodextrin", Electrophoresis 2005, *26* 3910-3920.