

R. Pootrakronchai, M. Kaewklum, P. Wilairat, T. Kusamran and T. Anukarahanonta

Method Validation for *d,l*-Isomers of Methamphetamine and Amphetamine by GC/MS

National Doping Control Centre, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand

Introduction

Amphetamine and methamphetamine are potent central nervous system stimulants (1). Since 2006, the differentiation between *d*- and *l*-isomers of methamphetamine is required by WADA (2). There are a number of drugs that are metabolized in the body to amphetamine and/or methamphetamine: amphetaminil, benzphetamine, clobenzorex, selegiline, dimethamphetamine, ethylamphetamine, famprofazone, fencamine, fenethylline, fenproporex, furfenorax, mefenorex, mesocarb and prenylamine. Thus, it is important to separate the enantiomers of amphetamine (AP) and methamphetamine (MA) excreted in urine in order to determine the source of the originally administered drugs. The enantiomeric separation of AP and MA can be achieved using the chiral agent, (S)-(-)-N-trifluoroacetyl-propyl chloride (*l*-TPC), as derivatising agent (3,4). A GC/MS procedure for separating the *d,l*- enantiomer of MA and AP was developed and validated.

Experimental

Blank urine from 5 healthy volunteers were spiked with standard substances to the final concentration of 0.06-1.00 µg/ml. The separation of AP and MA enantiomeric pair was performed by the modified Procedure I (liquid-liquid extraction) (5). 50 µl of 0.1 M (S)-(-)-N-trifluoroacetyl-propyl chloride (*l*-TPC) in dichloromethane was added to the extracted organic phase. After 15 min at room temperature, they were washed with 3 ml of 0.01M NaOH for 15 min and centrifuged at 2500 rpm for 15 min. The organic layer was separated and dried under the N₂ stream. The residue was redissolved in 100 µl *tert*-butyl methyl ether and 2 µl were injected into the GC.

Instrumentation

Agilent GC/MSD (6890/5973); column 5% PHME Siloxane (Ultra2), (12.5 m, i.d 0.2 mm, film thickness 0.11 μm); split ratio 10:1; flow at 0.6 ml/min; solvent delay 1.2 min; temperature program, initial 70°C (0.13 min), 25°C/min to 300°C (hold 1.2 min); transfer line temperature 280 °C

Results and Discussion

Although m/z 166 is the base ion, it was not selected as the quantifier ion as the ion is derived from *l*-TPC. The quantifier ions for the derivatised AP and MA are m/z 237 and 251, respectively (4). Figures 1 and 2 show that this method can separate the enantiomers of MA and AP, respectively. An excretion urine for methamphetamine shows only *d*-AP, and *d*-MA peaks (Figure 3). An excretion urine for selegiline shows *l*-AP, and *l*-MA peaks (Figure 4). Thus separation and identification of the isomer peaks can be used to differentiate the source of the originally administered drugs.

Table 1 shows results from the method validation for the enantiomers of AP and MA. Overall recovery was 80%, with %CV less than 15% (a valid range for repeatability) (6) and the LOD was about 10 times less than the minimum required performance limits (MRPL) for the detection of prohibited substances (0.5 $\mu\text{g/ml}$) (7).

Table 1. Summary of results for method validation (n=5)

Substance	%Recovery	%CV	LOD (ng/ml)	RRT*
<i>d</i> -MA	78.63	2.58	60	1.534
<i>l</i> -MA	81.20	1.55	60	1.517
<i>d</i> -AP	72.94	2.33	60	1.398
<i>l</i> -AP	68.34	2.29	60	1.374

*ISTD = Diphenylamine, RT = 4.09 min

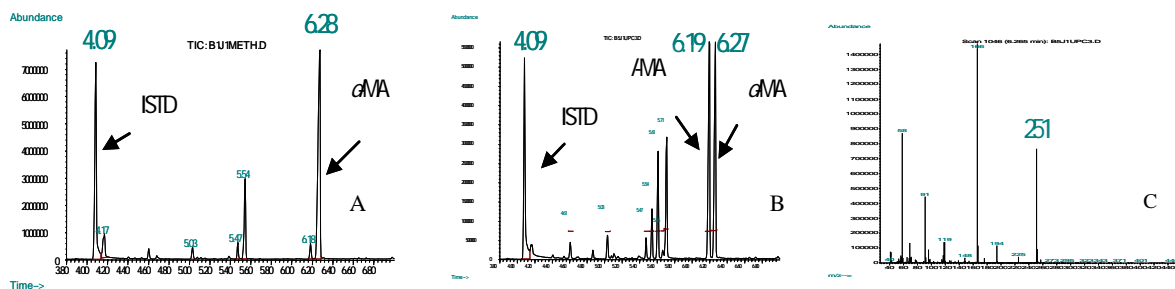


Figure 1. A. TIC of spike sample (1 µg/ml), *d*-MA-TPC (6.28 min), ISTD (RT= 4.09 min), B. TIC of spike sample (1 µg/ml), *l*- MA-TPC (6.19 min), *d*-MA-TPC (6.27 min), ISTD (RT=4.09 min), C. Mass spectrum of *d*-MA-TPC in spike sample (1µg/ml)

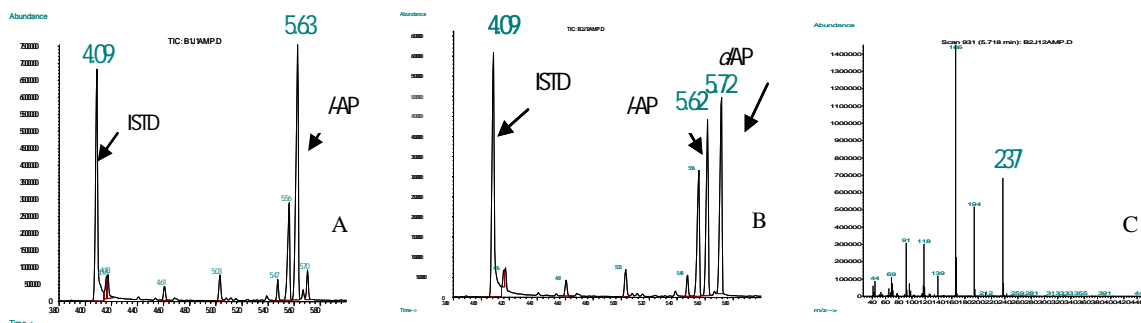


Figure 2. A. TIC of spike sample (1 µg/ml), *l*-AP-TPC (5.63min), ISTD (RT= 4.09 min), B. TIC of spike sample (1 µg/ml), *l*- AP-TPC (5.62 min), *d*-AP-TPC (5.72 min), ISTD (RT= 4.09 min), C. Mass spectrum of *d*-AP-TPC in spike sample (1µg/ml)

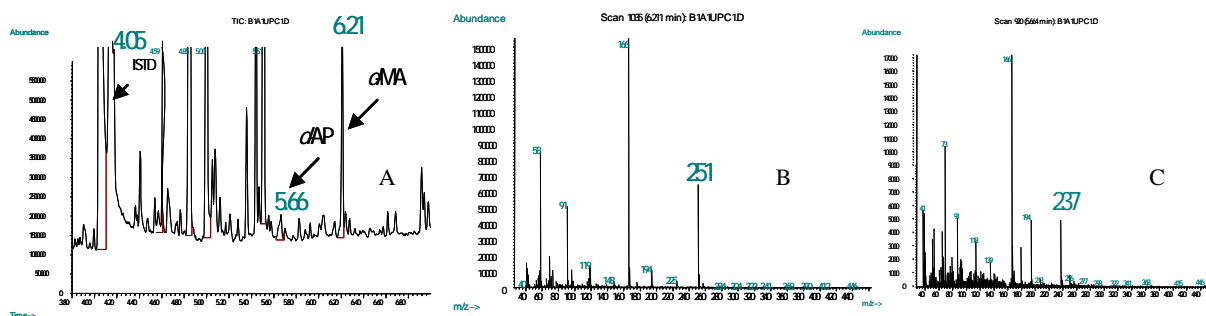


Figure 3. A. TIC of positive methamphetamine sample, B. Mass spectrum of *d*-MA-TPC (RRT= 1.533), C. Mass spectrum of *d*-AP-TPC (RRT= 1.397)

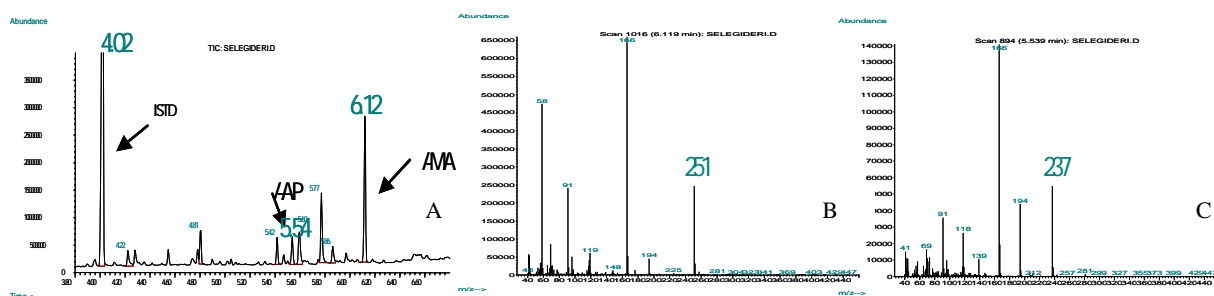


Figure 4. A. TIC of an excretion urine of selegiline, B. Mass spectrum of *l*-MA (RRT= 1.522), C. Mass spectrum of *l*-AP (RRT= 1.378)

References

1. Kramer, T., Maurer, HH. (1998) Determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine. *J Chromatogr B* **713**, 163-187.
2. World Anti-Doping Agency. The 2006 Prohibited List. International Standard, Montreal (2006) http://www.wada-ama.org/rtecontent/document/2006_LIST.pdf
3. John, TC. (1996) Enantiomeric composition of amphetamine and methamphetamine derived from the precursor compound famprofazone. *Forensic Sci Int.* **80**, 189-199.
4. Sheng, MW., Ting, CW., Yun, SG.(2005) Simultaneous determination of amphetamine and methamphetamine enantiomers in urine by simultaneous liquid-liquid extraction and diastereomeric derivatization followed by gas chromatographic-isotope dilution mass spectrometry. *J Chromatogr B* **816**, 131-143.
5. Hemmersbach, P., de la Torre, R. (1996) Stimulant, narcotics and β -blockers: 25 years of development in analytical techniques for doping control. *J Chromatogr B* **687**, 221-238.
6. Jimenez, C., Ventura, R., Segura, J. (2002) Validation of qualitative chromatographic methods: strategy in antidoping control laboratories. *J Chromatogr B* **767**, 341-351.
7. Minimum required performance limits for detection of prohibited substances. (2004) WADA technical document -TD2004MRPL version 1.0