T. Sobolevsky, I. Kachala, S. Bolotov, G. Rodchenkov

Detection of L- and D-stereoisomers of methamphetamine in urine following derivatization with menthyl chloroformate

Moscow Anti-Doping Centre, Moscow, Russia

Introduction

According to WADA Prohibited List, all anti-doping laboratories are required to discriminate between *L*- and *D*-stereoisomers of methamphetamine, as the former stereoisomer is considered as a 'specified substance' that is '*particularly susceptible to unintentional anti-doping rule violation because of their general availability in medicinal products* <…>' [1].

Chiral chromatographic separation of amphetamine-like compounds is usually achieved using N-trifluoroacetyl-L-prolyl chloride or α-methoxytrifluorophenylacetic acid (Mosher's acid) [2-4]. Here we report an alternative approach based on application of menthyl chloroformate for derivatization of methamphetamine stereoisomers. This reagent is cheap and preparation of chromatographically separable methamphetamine derivatives could be done easy.

Materials and Methods

Reagents: *D*-Methamphetamine and *L*-amphetamine were obtained from LGC Standards. (-)-Menthyl chloroformate (MeCF), *n*-pentane, pyridine and isooctane were purchased from Sigma-Aldrich. Potassium carbonate and hydrocarbonate were from Chimmed, Russia. All solutions were prepared using Milli-Q water.

Sample preparation: to 1 ml of urine internal standard is added (1000 ng amphetamine), followed by addition of 0.25 ml of carbonate buffer (K_2CO_3/$KHCO_3$, 100 g/l each), 20 µl of pyridine and 50 µl of 2% MeCF solution in isooctane. After brief vortexing, 5 ml of *n*-pentane are added to extract the derivatives by shaking for 10 min. After 5-min centrifugation at 2500 rpm the organic layer is evaporated to dryness at reduced pressure and reconstituted in 100 µl of ethyl acetate.

GC-MS:

Instrument 1: Agilent 5973 MSD, column Restek Rxi-1ms 12 m × 0.20 mm × 0.33 µm, carrier gas helium at 0.6 ml/min, injection of 2 µl with split 20:1 at 280°C, temperature program: 160°C (0 min), 1°C/min to 180°C (0 min), 40°C/min to 300°C.

Instrument 2: Thermo Finnigan DSQ II, column Restek RTX-35ms 30 m × 0.25 mm × 0.25 µm, carrier gas helium at 1.0 ml/min, injection of 2 µl with split 20:1 at 280°C, temperature program: 150°C (0 min), 10°C/min to 210°C (0 min), 5°C/min to 255°C, 40°C/min to 300°C.

Data were acquired in fullscan mode.

Results and Discussion

Menthyl chloroformate, being optically active itself, provides an opportunity to separate chiral compounds on a common gas chromatographic stationary phase. We applied a procedure described in [5] but sample preparation procedure was essentially modified.

The reaction of methamphetamine with menthyl chloroformate proceeds under catalytic influence of pyridine as follows (Fig. 1):

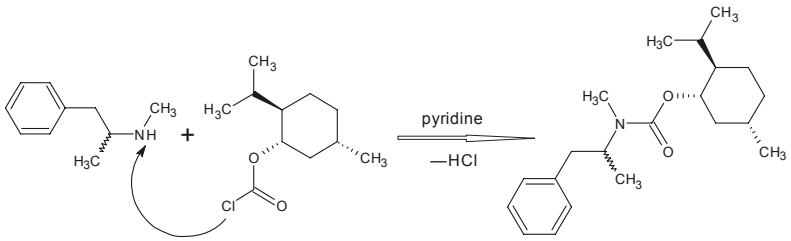

Fig. 1. Menthoxycarbonylation of amines (exemplified by methamphetamine)

The menthoxycarbonyl derivatives are stable, possess good chromatographic properties and have characteristic mass spectra. However, a number of derivatization artifacts (menthol, bis-menthyl carbonate *etc.*) are generated in course of the reaction but they do not interfere with derivatives in question. Amphetamine reacts in the similar way; only one hydrogen atom is substituted for menthoxycarbonyl group. It is worth saying that *L*- and *D*-amphetamine stereoisomers cannot be chromatographically separated as menthoxycarbonyl derivatives.

Electron ionization mass spectra of methamphetamine and amphetamine menthoxycarbonyl derivatives are shown in Fig. 2 and Fig. 3.

Methamphetamine stereoisomers were baseline separated on a chromatographic column with mid-polarity stationary phase (35% phenyl- 65% dimethylpolysiloxane), as demonstrated in Fig. 4. As one can see, this kind of derivatives provides excellent selectivity in detection of methamphetamine.

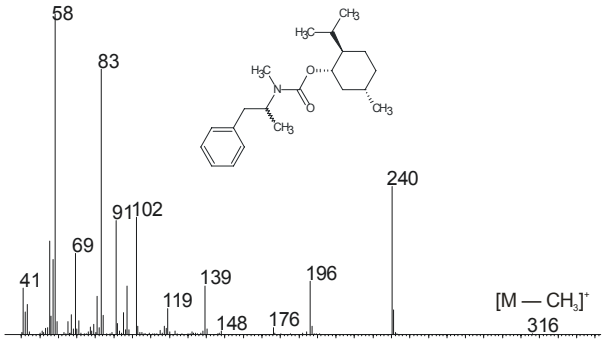

Fig. 2. Electron ionization mass spectrum of methamphetamine, N-menthoxycarbonylate.

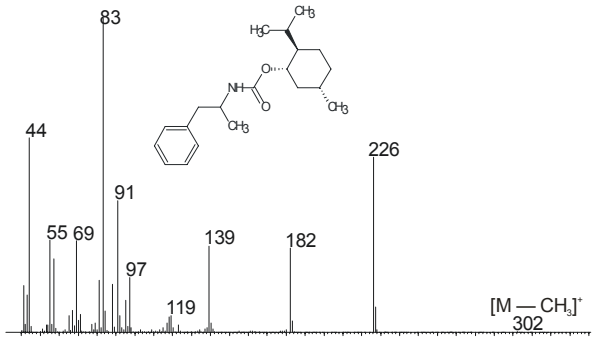

Fig. 3. Electron ionization mass spectrum of amphetamine, N-menthoxycarbonylate.

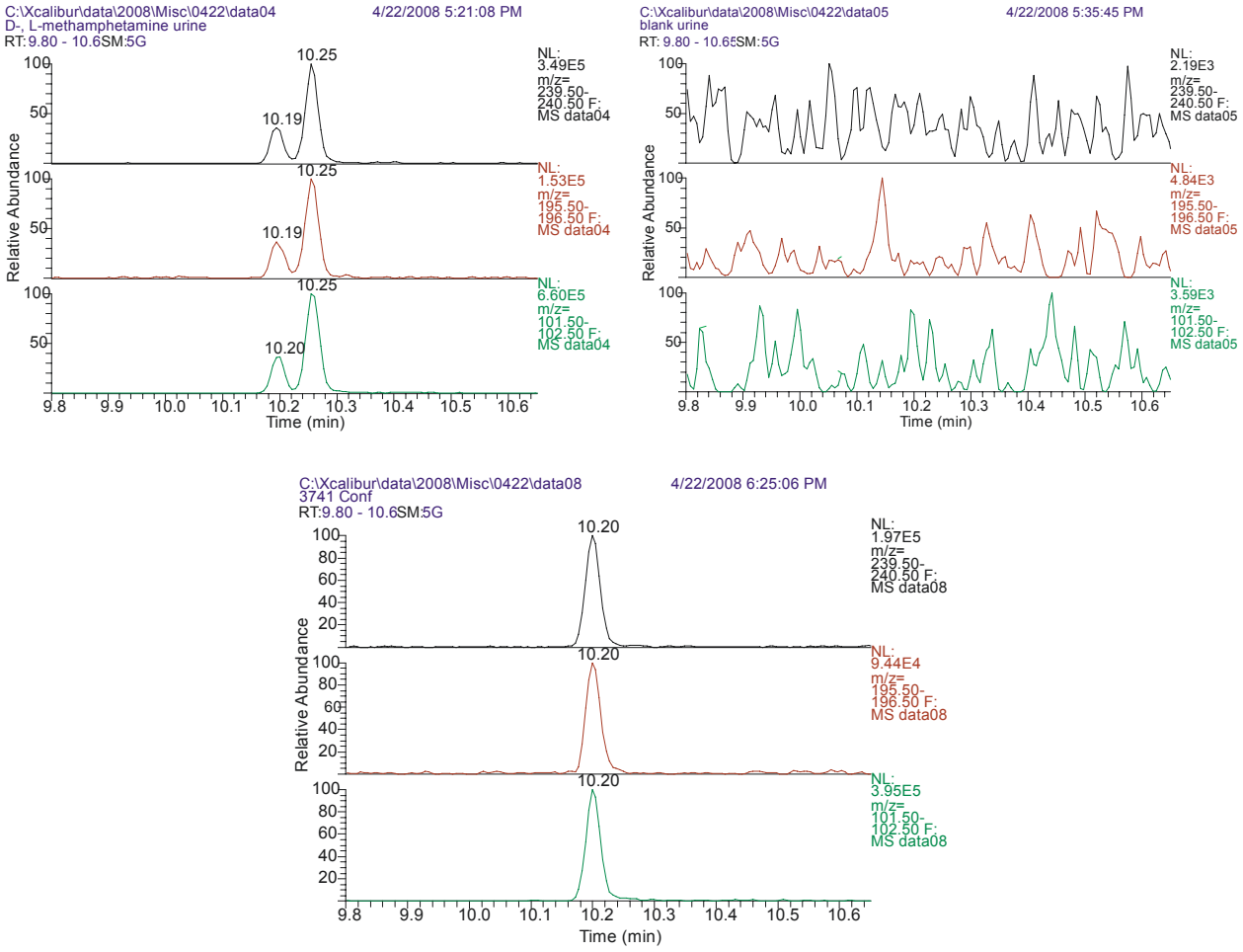

Fig. 4. Mass chromatograms (from left to the right) for excretion urine, blank urine and a WADA PT sample containing *D*-methamphetamine plotted against *m/z* 240, 196, 102.

However, it is not always appropriate to have a dedicated instrument with mid-polarity column. Reasonable separation could be achieved even using common non-polar chromatographic column with 100% polydimethylsiloxane stationary phase (see Fig. 5).

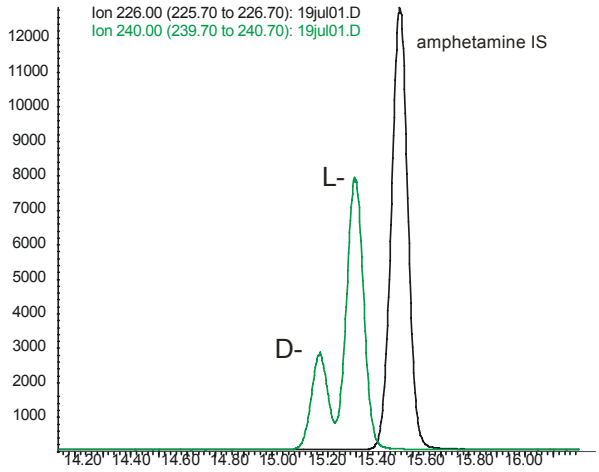

Fig. 5. Mass chromatogram plotted against ions *m/z* 240 (methamphetamine) and *m/z* 226 (amphetamine ISTD) in a routine sample

Conclusion

The menthoxycarbonyl derivatives of methamphetamine can be easily prepared and are suitable for separation of *L*- and *D*-methamphetamine stereoisomers. The derivatives can be baseline separated on a middle polarity stationary phase. However, even a non-polar column still gives enough resolution to unequivocally discriminate between both stereoisomers within a 20-min run. Detection limit of this procedure is *ca*. 10-50 ng/ml depending on urine sample aliquot volume.

References

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