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A rapid method for the extraction and enantiomeric separation of amphetamine-type stimulants

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Introduction

Amphetamines and designer drugs are a class of substances widely used among young people; their determination in biological specimens is therefore a main issue in forensic toxicology and in antidoping analysis, being these substances prohibited in competition. According to WADA rules, confirmation must be performed in order to obtain a fragmentation pattern with at least three diagnostic fragments with an abundance greater than 5% [1]. It is also required to distinguish between D- and L- isomers of methamphetamine due to their distinction in “non specified” and “specified” substances [2]; unambiguous identification of specific isomers can be extremely useful also for amphetamine, and, to a lesser extent, also for the other related substances (MDA, MDMA, MDEA), as the enantiomeric analysis can allow to draw conclusion with respect to both the origin and the metabolism of the target drug. The confirmation of amphetamines is generally performed using as derivatizing agent pentafluoropropionic, trifluoroacetyc or heptafluorobuthyrric anhydride, or MSTFA/MBTFA. For the enantiomeric separation, capillary electrophoresis has been proposed for these substances [3], but this procedure still needs a further confirmation by mass spectrometry. Chiral derivatizing agents have been proposed for amphetamine determination, such as Mosher’s acid chloride [4], or N-heptafluorobutyryl-4-heptafluorobutyloxy-prolyl chloride, synthesized “in house” by the laboratory performing the analysis [5]. N-Trifluoroacetyl prolyl chloride (TFAPC) has been proposed for the analysis of amphetamine and methamphetamine [6]. Here we present a simple and rapid method for the confirmation and enantiomeric separation of amphetamine, methamphetamine, MDA, MDMA and MDEA.

Materials and Methods

R- and S- amphetamine, R and S-methamphetamine, (±) amphetamine, (±) methamphetamine, (±) MDA, (±) MDMA, (±) MDEA and (±) MBDB were obtained from LGC Standards (Milano, Italy); S-(-)N-(trifluoroacetyl)prolyl chloride (0.1M in dichloromethane) (TFAP), and Diphenylamine were supplied by Sigma-Aldrich (Milano, Italy).

Sample preparation

To 2 mL of urine was added as Internal Standard diphenylamine (1 μg/mL) and the pH was adjusted to 9.5 with saturated carbonate buffer; then 4 ml of hexane and 50 μL of TFAP were added to the tube, and the samples were directly extracted in a mechanical shaker for 5 min. After centrifugation at 3000 g for 3 min, the organic layer was evaporated to dryness, reconstituted in 50 μL of ethyl acetate and 1 μL directly injected in GC/MS.

GC/MS conditions

The GC/MS system was an Agilent HP6890 gas chromatographer coupled to a 5973 mass spectrometric detector. Chromatographic conditions were the following: G&W Scientific Agilent Tecnologies 5% phenyl-methylsilicone capillary column (17m x 0.2 mm i.d., 0.33 μm film thickness); the oven temperature was held at 100°C for 1 min, increased to 140 at 8°C/min., to 310°C at 30°C/min (held 2 min.). The injection port was set at 270°C in splitless mode (purge time 1 min), and helium was used as carrier gas at a constant pressure of 20 psi. The mass detector operated in electron impact ionization at 70 eV in full scan mode (mass range: 50-430 m/z)

Results

All the amphetamines considered can be identified with an adequate fragmentation pattern, and isomers are well separated chromatographically. The retention times of the R and S enantiomers are respectively: amphetamine 10.07 and 10.16 minutes, typical fragments m/z 166, 194, 237; methamphetamine 10.65 and 10.72 minutes, typical fragments m/z 166, 58, 251, MDA 11.27 and 11.38 minutes, typical fragments m/z 162, 237, 372, MDMA 11.78 and 11.83 minutes, typical fragments m/z 162, 58, 386 and MDEA 11.84 and 11.92 minutes, typical fragments m/z 162, 72, 400. In mixture, S-MDMA and R-MDEA co-elute, but they can be identified by their typical mass fragments. Except for amphetamine and

methamphetamine, the spectrum presents the molecular ion, that helps to ensure the identification of the compound. No interfering peaks were detected at the expected retention times of the analytes of interest in ten negative urine samples. Related drugs and anorexic compounds (phenmetrazine, fentermine, fenfluramine, propilhexedrine,, \ethylamphetamine, MBDB, ephedrines) do not interfere with studied amphetamines, but could, in principle, be confirmed with the present method. The Limit of Detection (LOD) was determined at 25 ng/mL for amphetamine and methamphetamine and 50 ng/mL for MDA, MDMA and MDEA, far lower than MRPL [7]. The method is linear in the range 50-2000 ng/mL and with an acceptable repeatability (CV% less than 10% at 50 ng/ml). The method has therefore been applied on three real samples from antidoping controls with suspected amphetamines and demonstrated the presence of both R(-) and S(+) isomers of amphetamine in one case, of S(+)- methamphetamine in another, and of R(-) amphetamine and R(-) methampethamine in a sample showing also the presence of selegiline and its dealkylated metabolite. Figure 1 shows the chromatogram of a blank urine spiked with a mixture of the amphetamines and congeners studied at 500 ng/ml (MRPL). Figure 2 reports the typical mass spectra obtained.

In conclusion, the presented method allows the simultaneous identification MS of all the amphetamines and congeners, by a fast and simplified sample pre-treatment procedure. All isomers are well resolved chromatographically and the respective fragmentation pattern allows the confirmation of the studied substances according to WADA rules.

References

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Fig. 1. Urine spiked with a mixture of (±) amphetamine, (±) methamphetamine, (±) MDA, (±) MDMA, (±) MDEA at 500 ng/ml.