SCREENING FOR THE PRESENCE OF PARA-METHYLTHIOAMPHETAMINE IN URINE BY SOME COMMERCIAL IMMUNOASSAYS AND CONFIRMATION BY GC/MS

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ABSTRACT: *Para*-methylthioamphetamine (MTA) is a relatively new sulphur containing phenylalkylamine designer drug which was found in so-called S-5 tablets sold in Dutch smart shops. In this study, we evaluated the detection of MTA in urine specimens using commercial amphethamine-like immunoassays. For confirmation, MTA was analysed by GC/MS using different derivatisation methods.

KEY WORDS: p-Methylthioamphetamine; Immunoassay; GC/MS.

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INTRODUCTION

Para-methylthioamphetamine (MTA) is a relatively new sulphur containing phenylalkylamine designer drug. It is found in so-called S-5 tablets which are sold in Dutch smart shops [1]. In the Netherlands, one death related to MTA was reported and it is also thought to be responsible for three deaths in the United Kingdom. Only in one case no other drugs were involved. In these intoxication cases, significant amounts of the parent drug were found in urine [2].

The aim of this study is to evaluate the detection of MTA in urine specimens using commercial amphetamine-like immunoassays and the confirmation of the designer by GC/MS.

MATERIALS AND METHODS

Materials

Acetonitrile was purchased from Biosolve B. V. (Valkenswaard, the Netherlands). Concentrated hydrochloric acid (37% w/v) and methanol were purchased from Merck

Nederland (Amsterdam, the Netherlands). Trifluoroacetic anhydride (TFAA) and heptafluorobutyric anhydride (HFBA) were obtained from Aldrich (Zwijndrecht, the Netherlands). The amphetamine/methamphetamine II reagent cartridge, calibrators, controls and reagents were obtained from Abbott Diagnostics (Hoofddorp, the Netherlands). The EMIT® d.a.u.™ monoclonal amphetamine/methamphetamine reagents, the EMIT® d.a.u.™ amphetamine class reagents, calibrators and buffer were obtained from Behring Diagnotics (Rijswijk, the Netherlands). All other reagents and chemicals were of analytical grade.

Immunoassays

Blank urine was spiked with concentrations of MTA ranging from 3.10^{-5} mM to 100 mM. For comparison amphetamine, dexamphetamine and methamphetamine were tested as well.

The samples were analysed using the fluorescent polarization immunoassay (FPIA) amphetamine/methamphetamine II assay on the Abbott ADx® analyzer and the enzymatic immunoassays EMIT® d.a.u.™ monoclonal amphetamine/methamphetamine assay and the EMIT® d.a.u.™ amphetamine class assay on the Syva ETS® analyzer. Analysis were performed according to the manufacturer's directions.

GC/MS analyses

After addition of a few drops of concentrated hydrochloric acid (37% w/v) to 10 μg of MTA, amphetamine, and methamphetamine in methanol, the samples were evaporated to dryness under nitrogen at 60°C. Derivatization of MTA, amphetamine, and methamphetamine into *N*-TFA or *N*-HFB derivative was performed by addding 50 μl of acetonitrile and 50 μl TFAA or HFBA respectively, to the dry sample residues. The mixture was heated for 20 min at 60°C. Excess of reagent was removed under nitrogen at 50°C and finally the residue was redissolved in 100 μl of acetonitrile.

GC/MS analysis was performed on a Hewlett Packard 5790A gas chromatograph equipped with a HP Ultra 1 column (16.5 m x 0.20 mm, 0.11 mm film thickness) coupled to a Hewlett Packard 5970A mass selective detector and a Hewlett Packard 7673A automatic sampler (Hewlett Packard, Palo Alto, CA, USA). The injection volume was 1 μl. The operating temperatures for the GC were 280°C for the injector and the oven was programmed from 80°C (1 min) to 290°C (10 min) at 10°/min. The carrier gas was helium. The mass spectra were taken full scan in the electron ionization (EI) mode (m/z 50 to 600). Retention index (RI) values were obtained using a homologous series of hydrocarbons.

RESULTS AND DISCUSSION

Immunoassays

Figures 1 and 2 show the curves of log concentration versus absorbance using the EMIT® d.a.u.™ monoclonal amphetamine/methamphetamine assay and the EMIT® d.a.u.™ amphetamine class assay. Figure 3 shows the curve of log concentration versus net polarization using the FPIA amphetamine/methamphetamine II assay. The results demonstrate that MTA cross-reacts with the antibodies of the EMIT® monoclonal assay, the EMIT® class assay and the FPIA assay, although with different affinities.

GC/MS analyses

Fig. 1. Curve of log concentration versus absorbance using the EMIT monoclonal assay.

The EI mass spectra of non-derivatized and the N-TFA and the N-HFB derivatives of MTA are shown in figures 4, 5 and 6, respectively. The mass spectra of the MTA derivatives seem to be more characteristic than the mass spectrum of non-derivatized MTA. The RI of non-derivatized and the N-TFA and the N-HFB derivatives are presented in Table I. For comparison the RI values for amphetamine and methamphetamine and their derivatives are included.

TABLE I. RI OF NON-DERIVATIZED AND THE *N*-TFA AND THE N-HFB DERIVATIVES OF MTA, AMPHETAMINE AND METHAMPHETAMINE

MTA	1515
Amphetamine	1135
Methamphetamine	1176
MTA-N-TFA	1672
Amphetamine-N-TFA	1281
Methamphetamine-N-TFA	1378
MTA-N-HFB	1702
Amphetamine-N-HFB	1338
Methamphetamine-N-HFB	1424

CONCLUSION

The presence of MTA in urine can be detected by the respective commercial immunoassays and confirmed by GC/MS.

Fig. 2. Curve of log concentration versus absorbance using the EMIT class assay.

Fig. 3. Curve of log concentration versus net polarization using the FPIA assay.

Fig. 4. Spectrum of MTA.

References:

1. de Boer D., Egberts T., Maes R. A. A., Para-methylthioamphetamine, a new amphetamine designer drug of abuse, *Pharmacy, World and Science* 1999, vol. 21, pp. 47–48.

Fig. 5. Spectrum of MTA-N-TFA.

Fig. 6. Spectrum of MTA-N-HFB.

2. European Monitoring Centre for Drugs and Drug Addiction, Report on the risk assessment of 4-MTA in the framework of the joint action on new synthetic drugs, 1999.