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Determination of amphetamine-derived designer drugs in human urine by SPE extraction and capillary electrophoresis with mass spectrometry detection

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Abstract

In recent years, a number of newer designer drugs have entered the illicit drug market. The methylenedioxy-derivates of amphetamine represent the largest group of designer drugs. This paper describes a method for screening for and quantification of ten 2,5-methylenedioxy-derivates of amphetamine and phenylethylamine in human urine, using capillary electrophoresis coupled to electrospray ionisation–mass spectrometry (CE–ESI–MS). Prior to CE–MS analysis, a simple solid-phase extraction (SPE) was used for sample cleanup. The method was validates according to international guidelines.

Keywords: Methylenedioxy-derivates; Amphetamine; Phenylethylamine; Designer drug; Urine; Capillary electrophoresis; Mass spectrometry; Solid-phase extraction (SPE)

1. Introduction

In the last few decades, the amphetamine designer drugs have gained popularity as recreational drugs and are used mainly for their agreeable stimulating effects, especially in gatherings known as “raves” [1]. The popularity of the methylenedioxyamphetamine derivates can be attributed to their psychotropic effects and the so-called entactogenic effects [2].

The methylenedioxy-derivates of amphetamine represent the largest group of designer drugs. The term “designer drug” includes compounds that have been chemically altered from federally controlled substances in order to bypass the legal regulations and to produce more potent substances. Examples are derivates with one or two methoxy groups over the phenyl-ring, with halogens, sulphur and methyl group attached against each other [3].

Up until now, nearly 200 different derivates have been synthesised and described by Shulgin and Shulgin [4]. Only a limited number of these derivates are known from Europe, although both halogen and sulphur-derivates have been detected in confiscated tablets or biological samples [5–7].

Monitoring of amphetamines and designer drugs in human urine is successfully used for clinical and forensic application and in surveillance of drug substitution. To date, the determination of amphetamines in urine samples has been mainly on GC–MS [8–12] and HPLC–DAD [7,13–14]. In the last few years, the liquid chromatography coupled mass spectrometry (LC–MS) has developed rapidly in forensic and clinical applications as well as in analysis of amphetamines in biological samples [15–17]. On the other hand, the on-line combination of capillary electrophoresis (CE) and mass spectrometry (MS) has been established as a powerful method for forensic urine samples screened [18–21].

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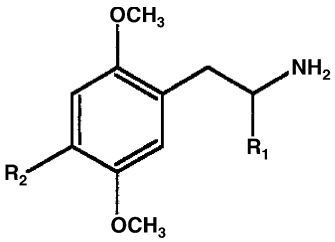

	Compounds	R_1	R_2
1	2,5-dimethoxy-amphetamine HCl	CH_3	H
2	2,5-dimethoxy-4-methyl-phenethylamine HCl	H	CH_3
3	2,5-dimethoxy-4-methyl-amphetamine HCl	CH_3	CH_3
4	2,5-dimethoxy-4-chloro-amphetamine HCl	CH_3	Cl
5	2,5-dimethoxy-4-nitro-phenethylamine HCl	H	NO_2
6	2,5-dimethoxy-4-nitro-amphetamine HCl	CH_3	NO_2
7	2,5-dimethoxy-4-bromo-phenethylamine HCl	H	Br
8	2,5-dimethoxy-4-bromo-amphetamine HCl	CH_3	Br
9	2,5-dimethoxy-4-iodo-phenethylamine HCl	H	I
10	2,5-dimethoxy-4-iodo-amphetamine HCl	CH_3	I

Fig. 1. Chemical structures of amphetamine derivates studied in this work.

This paper describes a method for screening for and quantification of ten 2,5-methylenedioxy-derivates of amphetamine and phenylethylamine (Fig. 1) in human urine, by capillary electrophoresis coupled to electrospray ionisation–mass spectrometry (CE–ESI–MS). Using an aqueous pH 4.5 buffer composed of ammonium acetate/acetic acid, the CE–MS analysis provided data that permitted the unambiguous confirmation of these drugs in human urine.

This procedure is simple, clean and should be easily applied to epidemiological and clinical studies. In addition, the mass spectrum of these amphetamine derivates can be useful for future their identification with CE–MS in biological matrices as well as in confiscated tablets.

2. Experimental

2.1. Materials

The 2,5-dimethoxy-derivates of amphetamine and phenylethylamine (Fig. 1) was synthesised in our laboratory at their maximum level of purity using a slight modification of a method described in literature [4]. The product characterisation by ^1H and ^{13}C NMR spectrometry was carried out using a Bruker AMX 500. Melting points (mp) were determined with a Kofler hot stage microscope. IR spectra were carried out using a Perkin-Elmer 1760-X IFT.

Deionised and distilled water was purified through a Milli Q water system (Millipore). Other reagents and solvents used were purchased at the highest commercial quality. Bond Elut C_{18} solid-phase extraction (SPE) columns (100 mg/ml) were purchased from Alltech (Italy) and mounted on a VacElut vacuum manifold (Supelco, USA).

Aqueous stock solutions (1.0 mg/ml) of amphetamine derivates were prepared, stored at −20 °C, and diluted with Milli Q water to appropriate concentrations before use.

Drug-free urine collected from a healthy adult male was used to make blank and spiked samples containing amphetamine derivates. The urine samples were kept frozen at −20 °C until analysed.

2.2. CE–electrospray ionisation (ESI)–MS set up

Separations in capillary electrophoresis were performed using a model Hewlett-Packard^{3D} CE system coupled with at Agilent 1100 series LC/MSD (Agilent Technologies) via an electrospray ionisation interface.

Table 1
Validation parameters of the method

Compound	Linearity range (ng/ml)	Correlation coefficient (r^2)	LOD (ng/ml)	LOQ (ng/ml)	Mean recovery (%)
2,5-Dimethoxy-amphetamine HCl	5.0–1000	0.9991	1.20	4.00	98
2,5-Dimethoxy-4-methyl-phenethylamine HCl	5.0–1000	0.9999	0.31	1.00	84
2,5-Dimethoxy-4-methyl-amphetamine HCl	1.0–1000	0.9996	3.87	12.90	82
2,5-Dimethoxy-4-chloro-amphetamine HCl	1.0–1000	0.9995	1.30	4.40	97
2,5-Dimethoxy-4-nitro-phenethylamine HCl	1.0–1000	0.9999	2.90	9.80	83
2,5-Dimethoxy-4-nitro-amphetamine HCl	1.0–1000	0.9999	4.29	13.98	80
2,5-Dimethoxy-4-bromo-phenethylamine HCl	1.0–1000	0.9999	1.60	5.30	82
2,5-Dimethoxy-4-bromo-amphetamine HCl	1.0–1000	0.9998	3.01	9.00	96
2,5-Dimethoxy-4-iodo-phenethylamine HCl	1.0–1000	0.9999	3.68	12.01	96
2,5-Dimethoxy-4-iodo-amphetamine HCl	1.0–1000	0.9996	2.00	6.50	81

Uncoated fused-silica capillary (120 cm × 75 μm i.d.) was used for the electrophoresis separation. A 75 μm capillary was chosen because a highest sensitivity is achieved in comparison to the 50 μm capillary, but still the required resolution of the compounds was maintained. The volatile buffer consisted of 50 mM ammonium acetate adjusted pH 4.5 with acetic acid. A separation voltage of 25 kV was applied. Samples were injected hydrodynamically with a pressure of 50 mbar for 10 s.

The following mass spectrometry conditions were used: capillary temperature, 200 °C; source-voltage, −3.5 kV; full scan, 50–500 *m/z*; positive ion mode; sheath gas 20 arbitrary units nitrogen. Sheath liquid consisting of acetonitrile–water–acetic acid (50:49.5:0.5).

2.3. Extraction procedure

A urine sample (1 ml) was mixed with hydrogencarbonate buffer (100 mM, pH 10, 1 ml). The mixture was applied to an Bond Elut C_{18} extraction column and forced to pass through at 1 ml/min by applying reduced pressure. The column has previously been activated and conditioned with 1 ml of methanol and 1 ml of 100 mM hydrogencarbonate buffer (pH 10). After application of the sample, the column was washed with 2 ml of Milli Q water and dried by passing a stream of air for 5 min. The analytes were then eluted with 2 ml of methanol and the eluate was evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 1 ml of the separation buffer.

2.4. Method validation

The method was validated according to international guidelines. To construct calibration curves, urine samples spiked with amphetamine derivates at concentrations ranging from 1.0 to 1000 ng/ml were prepared and analysed using the above procedure. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as the concentrations obtained multiplying the standard deviation of the range by three and ten times, respectively [22]. Recovery values were studied by spiking urine samples at three fortification levels (25–100–500 ng/ml) and analysing six replicates.

Reproducibility was evaluated by analysing samples containing 500 ng/ml of each amphetamine on the same day in

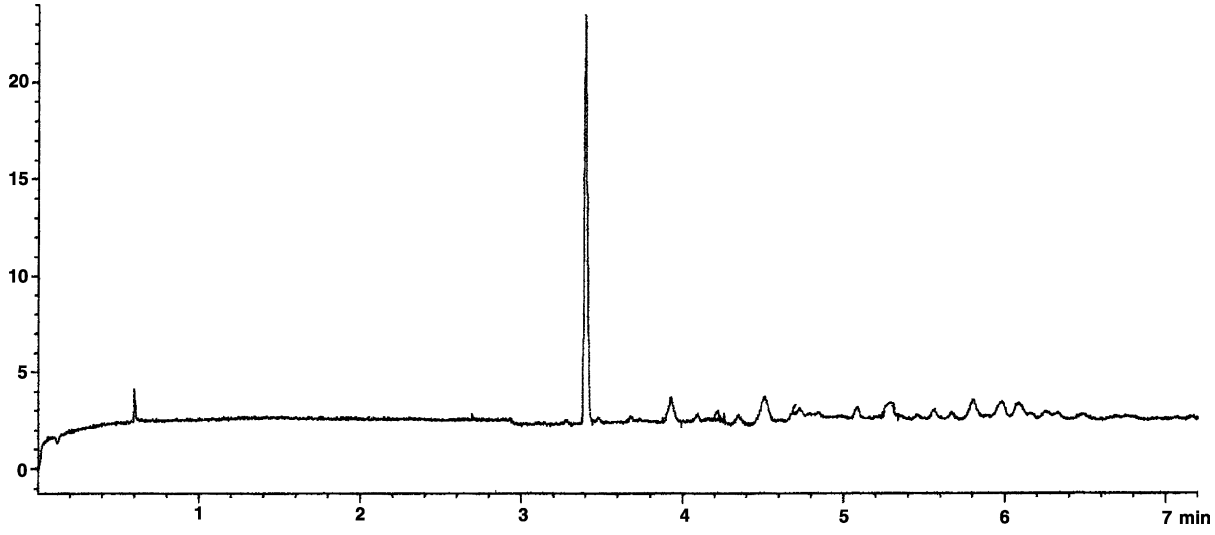

Fig. 2. Extract of urine blank.

five replicates (intra-day reproducibility) and over five consecutive days in duplicate (inter-day reproducibility). Finally, repeatibility of the instrument was evaluated calculating the migration time of a standard solution (500 ng/ml) of each amphetamine every 10 sample injection (with washing every third injection).

3. Results and discussion

The calibration curves showed linearity in the range of 5.0–1000 ng/ml for all amphetamines analysed and the correlation coefficients (r^2) were higher than 0.9991 (Table 1). The limits of detection (LOD) and quantification (LOQ) ranged

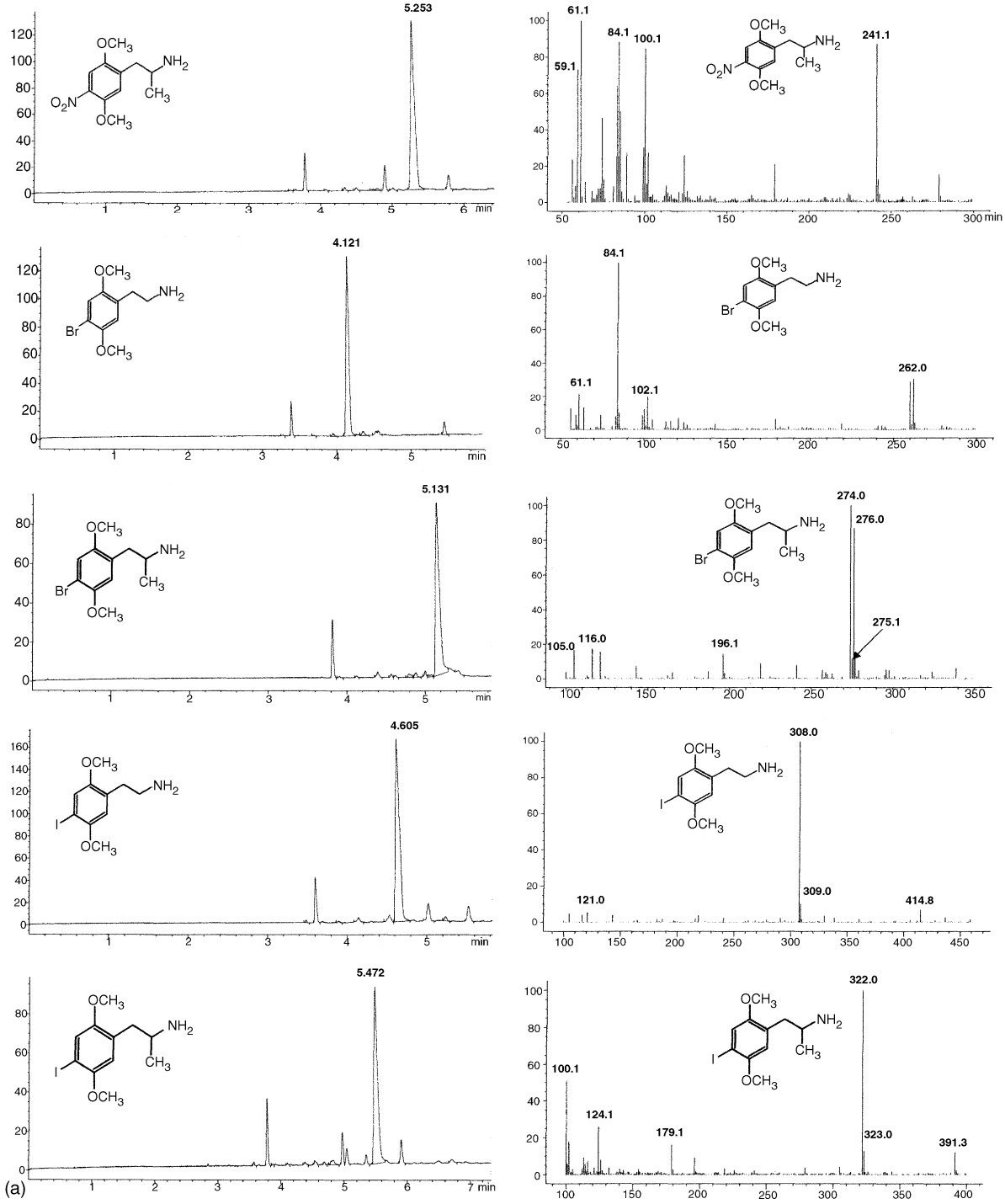

Fig. 3. Extracts of urine samples spiked with amphetamine derivates and MS-spectra relative to 10 amphetamines analyzed.

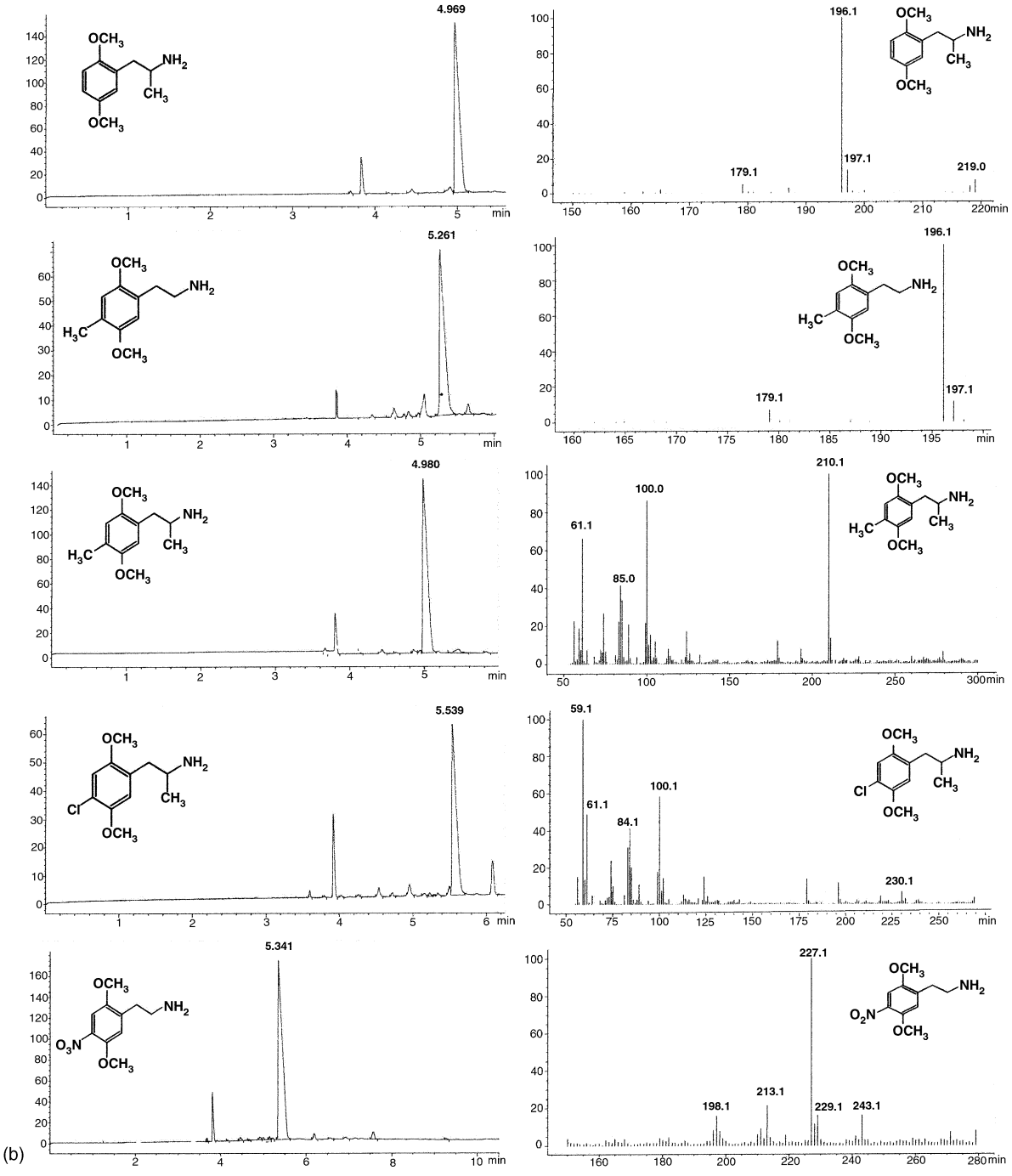

Fig. 3. (*Continued*).

from 0.31 to 4.29 ng/ml and from 1.00 to 13.98 ng/ml, respectively (Table 1).

This sensitivity was comparable to that observed by CE with mass spectrometry detection for similar amphetamines [18] and was sufficient for confirmatory testing of urinary levels of drug consumers.

Recoveries obtained from spiked urine were better than 80%. The means of recoveries at three fortification levels were reported in Table 1.

The extractive procedure from urine allows one to obtain electropherograms free of interfering extraneous peaks (Fig. 2). Typical electropherograms extracted from urine were showed in Fig. 3, where no interference from endogenous substances was observed.

Data for accuracy and precision (Table 2) were within required limits.

The CE–MS analysis allow the quantitative determination of amphetamine derivates comparable in sensitivity,

Table 2
Accuracy, intra- and inter-day precision for analysis of amphetamines

Compound	Reproducibility intra-day (S.D. × 10^{-2})	Reproducibility inter-day (S.D. × 10^{-2})	Repeatability of instrument (S.D., s)
2,5-Dimethoxy-amphetamine HCl	0.73	4.15	0.48
2,5-Dimethoxy-4-methyl-phenethylamine HCl	0.95	4.26	0.90
2,5-Dimethoxy-4-methyl-amphetamine HCl	1.10	4.16	2.10
2,5-Dimethoxy-4-chloro-amphetamine HCl	0.79	3.95	1.32
2,5-Dimethoxy-4-nitro-phenethylamine HCl	0.20	2.20	0.12
2,5-Dimethoxy-4-nitro-amphetamine HCl	0.16	3.55	0.18
2,5-Dimethoxy-4-bromo-phenethylamine HCl	1.25	2.20	1.14
2,5-Dimethoxy-4-bromo-amphetamine HCl	1.30	3.15	1.80
2,5-Dimethoxy-4-iodo-phenethylamine HCl	0.84	2.15	0.06
2,5-Dimethoxy-4-iodo-amphetamine HCl	0.15	2.30	0.36

S.D., standard deviation.

accuracy and precision to LC–MS techniques [14,16].

The main advantages of the present method lie in its simple, clean and reliable SPE extraction of the amphetamine-derived designer drugs from human urine followed by their detection and quantification by CE–MS. Although substantial differences in migration times were noted between different amphetamines, CE–MS data permit unambiguous identification of 10 amphetamines analysed. In addition, the mass spectrum of 2,5-methylenedioxy-derivates of amphetamine and phenylethylamine (Fig. 3) can be useful for future their identification with CE–MS in biological matrices as well as in confiscated tablets.

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