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Determination of 4-alkyl 2,5 dimethoxy-amphetamine derivatives by capillary electrophoresis with mass spectrometry detection from urine samples

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Abstract

The methylenedioxy-derivatives of amphetamine represent the largest group of designer drugs. The 4-methyl (DOM), -ethyl (DOET) and -propyl (DOPR) derivatives of 2,5-dimethoxy-amphetamine (2,5-DMA) were found to possess quite similar serotonin receptor affinities [R.A. Glennon, D.L. Doot, R. Young, Pharmacol. Biochem. Behav. 14 (1981) 287.]. This paper describes a method to screen for and quantify DOM, DOET and DOPR in urine samples, 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 validated according to international guidelines. Data for accuracy and precision were within required limits. Calibration curves were generated ranging from 10 to 1000 ng/mL and correlation coefficients always exceeded 0.996.
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Keywords: Urine; 2,5-Dimethoxy-4-methylamphetamine (DOM); 2,5-Dimethoxy-4-ethylamphetamine (DOET); 2,5-Dimethoxy-4-propylamphetamine (DOPR); Capillary electrophoresis-mass spectrometry (CE-MS)

1. Introduction

In the last few decades, the frequent appearance of amphetamine designer drugs on the illicit drug market has been a matter of concern for all authorities involved. New amphetamine drugs are being introduced because these compounds are not covered by existing legislation [1]. Therefore, the new drugs cannot be considered illicit drugs until their names are implemented. Examples are derivatives with one or two methoxy groups over the phenyl-ring, with halogens, sulphur and methyl group attached against each other [2].

Till date, nearly 200 different derivatives have been synthesised and described by Shulgin and Shulgin [3]. One such derivative is 2,5-dimethoxy-4-methylamphetamine (DOM); this agent is known to be hallucinogenic in man [4]. Introduction of a methyl group at the 4-position of 2,5-DMA enhances potency by more than an order of magnitudine. Homologation of this alkyl group to ethyl (DOET) and propyl (DOPR) produces an increase in potency; further homologation to butyl (DOBU), decreases potency, and to amyl (DOAM) results in an agent that does not produce DOM-like stimulus effects. The relative potencies of these agents, as compared to 2,5-DMA, are: 2,5-DMA < DOM < DOET < DOPR > DOBU [5].

This paper describes a method for screening and quantification of 4-methyl (DOM), -ethyl (DOET) and -propyl (DOPR) derivatives of 2,5-dimethoxy-amphetamine (2,5-DMA) (Fig. 1) in urine samples by capillary electrophoresis coupled to electrospray ionisation-mass spectrometry (CE-ESI-MS).

The monitoring of amphetamine derivatives in urine specimens has found successful clinical and forensic application and has also been used for the surveillance of drug substitution. Till date, the determination of amphetamines in biological samples has been performed mainly on GC–MS [6–11], HPLC-DAD [12,13] and CE-DAD [14,15]. In the last few years, the liquid chromatography coupled mass spectrometry (LC–MS) has developed rapidly in forensic and clinical applications as well

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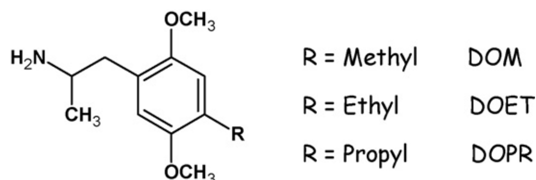

Fig. 1. Chemical structures of 2,5-dimethoxy-4-methylamphetamine (DOM), 2,5-dimethoxy-4-ethylamphetamine (DOET) and 2,5-dimethoxy-4-propylamphetamine (DOPR).

as in analysis of amphetamines in biological samples [16–18]. On the other hand, screened, the on-line combination of capillary electrophoresis (CE) and mass spectrometry (MS) has been established as a powerful method for forensic samples [19,20].

The CE-MS analysis provided data that permitted the unambiguous confirmation of these drugs in biological samples.

The extraction procedure is simple, clean and can easily be applied to epidemiological and clinical studies. In addition, the mass spectra of these amphetamine derivatives can be useful for their future identification with CE-MS in autopsy materials as well as in confiscated tablets.

2. Experimental

2.1. Materials

2,5-Dimethoxy-4-methylamphetamine hydrochloride (DOM), 2,5-dimethoxy-4-ethylamphetamine hydrochloride (DOET) and 2,5-dimethoxy-4-propylamphetamine hydrochloride (DOPR) were synthesised in our laboratory at their maximum level of purity using a method described in the literature [4]. The product characterisation by ^{1}H-NMR spectrometry was carried out using a Bruker AMX 400. Melting points (mp) were determined with a Kofler hot stage microscope. IR spectra were acquired 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 of the highest commercial quality. Bond Elut C_{18} solid-phase extraction columns (100 mg/ml) were purchased from Alltech (Italy) and mounted on a VacElut vacuum manifold (Supelco, USA).

Stock solutions (1.0 mg/ml) of DOM, DOET and DOPR hydrochlorides were prepared in Milli Q water, stored at +4 °C, and diluted to appropriate concentrations before use.

Drug-free urine collected from a healthy adult male was used to make blank and spiked samples containing amphetamine derivatives. The biological 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.

Uncoated fused-silica capillary (120 cm × 50 μm ID) was used for the electrophoresis separation. The volatile buffer consisted of 10 mM sodium phosphate monobasic adjusted to pH 4.5 with phosphoric acid.

A separation voltage of 10 kV was applied. Samples were injected hydrodynamically with a pressure of 25 mbar for 10 s.

In order to obtain the highest possible intensity for the quantification of ions, the fragmentation energy (cone voltage) was optimised. During this experiment, a mass range from *m/z* 100 to 300 was monitored in SCAN mode, applying different cone voltages. The SIM electropherograms of protonated molecular ions $[M+H]^+$ were acquired by selecting the ranges of $m/z \pm 0.5$ from the full scan mass spectra [21]. For the quantification of each compound, the protonated molecule was selected as the quantifier ion.

The following mass spectrometry conditions were used: capillary temperature 200 °C, source-voltage −4 kV, positive ion mode, sheath gas 20 arbitrary units nitrogen. Sheath liquid consisted of methanol-water-phosphate buffer 100 mM pH 4.5 (50:49.5:0.5).

2.3. Extraction procedure from urine

Amphetamines were extracted using our previously described method [19] as follows: 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 been activated previously 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 mobile phase.

2.4. Method validation

The analytical validation was performed according to international guidelines [22,23]. The specificity of the method was evaluated by analysing urine from 10 healthy non-drug-consuming subjects. Linearity was obtained with an average determination coefficient (r^2) >0.996. In order to construct calibration curves, urine samples spiked with amphetamine derivatives at concentrations ranging from 10 to 1000 ng/ml were prepared and analysed using the above described procedure. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as $y_{LOD} = b + 3SD_b$ and $y_{LOQ} = b + 10SD_b$, where b = intercept and SD_b = standard deviation of the intercept [24].

Repeatibility was evaluated by analysing samples containing 200 and 500 ng/ml of each amphetamine on the same day in five replicate (intra-day precision) and over five consecutive days in triplicate (inter-day precision) and by calculating the RSD (relative standard deviation) of the experimentally determined concentrations. Finally, repeatibility of the instrument was evaluated by calculating the migration time of a standard solution (200 ng/ml) of each amphetamine in 10 sample injections (with washing every third injection).

Table 1
Validation parameters of the method

Compound	Code	r^2	LOD (ng/ml)	LOQ (ng/ml)
2,5-Dimethoxy-4-methylamphetamine	DOM	0.997	3.98	13.80
2,5-Dimethoxy-4-ethylamphetamine	DOET	0.996	4.64	15.30
2,5-Dimethoxy-4-propylamphetamine	DOPR	0.998	4.36	14.10

The accuracy was expressed in terms of recovery percentage. Recovery values were studied by spiking urine samples at three fortification levels (25–100–500 ng/ml) and analysing six replicates.

3. Results and Discussion

The calibration curves showed linearity in the range of 10–1000 ng/ml for all amphetamines analysed and the correlation coefficients (r^2) were higher than 0.996 (Table 1). The limits of detection (LODs) and quantification (LOQs) ranged from 3.98 to 4.64 ng/ml and from 13.80 to 15.30 ng/ml, respectively (Table 1). This sensitivity was sufficient for confirmatory testing of urinary levels of drug consumers.

The extractive procedure from urine allows one to obtain electropherograms free from interfering extraneous peaks (Fig. 2A).

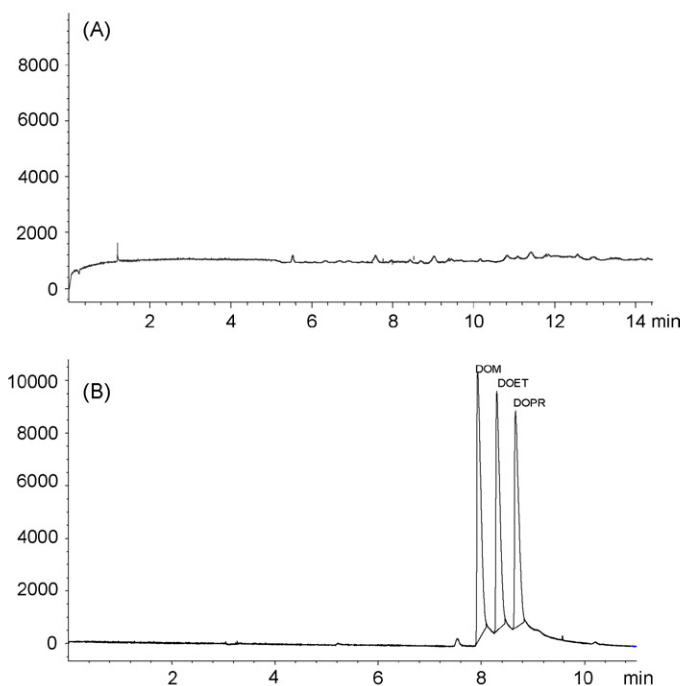

Fig. 2. Extract of blank urine (A) and urine sample spiked with DOM, DOET and DOPR (100 ng/ml) (B).

Table 2
Migration time and $[M+H]^+$ of amphetamines

Compound	t_M (min)	$[M+H]^+$
DOM	8.0	210
DOET	8.5	224
DOPR	8.9	238

Fig. 2B shows a full scan electropherogram of 100 ng/ml spiked urine, whereas the mass spectra of analytes are reported in Fig. 3.

Qualitative analysis was performed according to migration times (t_M) and relative mass spectra. The SIM mode was exercised for quantification, as described by Huang and Zhang [9]. The monitored ions and migration times are given in Table 2.

Data for precision and accuracy (Tables 3 and 4) were within required limits [23]. The intra-day and inter-day RSD% for two different concentrations (200 and 500 ng/ml) were from 1.32 to 4.30% (Table 3).

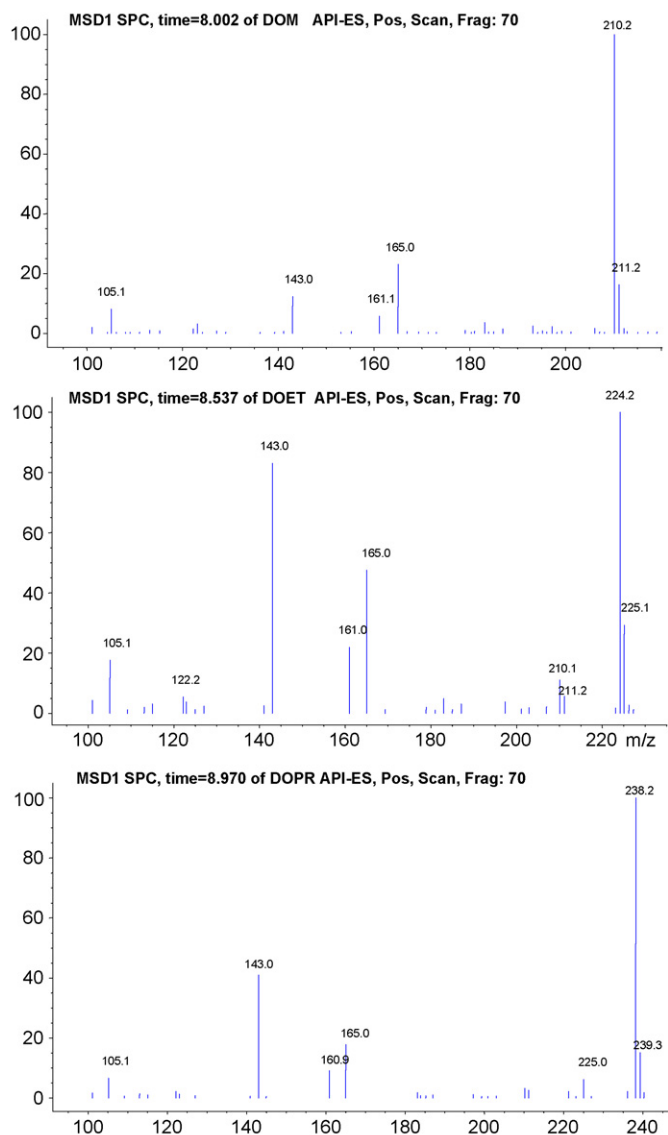

Fig. 3. Mass spectra of 2,5-dimethoxy-4-alkyl-amphetamine derivative.

Table 3
Intra- and inter-day precision for analysis of amphetamines

Compound	Concentration (ng/ml)	Repeatability intra-day RSD	Repeatability inter-day RSD	Repeatability instrument RSD
DOM	200	4.20	3.30	0.13
	500	1.48	4.10	
DOET	200	3.85	2.60	0.12
	500	1.32	4.52	
DOPR	200	2.77	3.21	0.09
	500	1.54	4.30	

RSD = relative standard deviation.

Table 4
Recovery

Compound	Spiked concentration (ng/ml)	Mean concentration (ng/ml)	Recovery (%)	Mean recovery (%)
DOM	25	19.5	75	83
	100	85.2	85	
	500	452.0	90	
DOET	25	17.7	71	78
	100	77.9	78	
	500	430.0	86	
DOPR	25	15.2	61	75
	100	80.0	80	
	500	424.8	85	

Recovery percentages obtained from spiked plasma were better than 75%. The recoveries were calculated by comparing the peak areas obtained from the extract of the spiked urine sample with those obtained by direct injection of standard solution. The values of recoveries at three fortification levels were reported in Table 4.

With regard to the analytical procedure, to the best of our knowledge, this is the first CE-MS method that enables the simultaneous determination of these compounds in human urine. Furthermore, CE-MS analysis allows for the quantitative determination of amphetamine derivatives and is comparable in sensitivity, accuracy and precision to GC–MS and LC–MS techniques [11,17].

The main advantages of our method is that it allows for simple, clean and reliable SPE extraction of amphetamine-derived designer drugs from urine samples. Another benefit is that CE-MS does not require sample derivatization prior to analysis to improve the sensitivity of the method. In addition, CE-MS data permit unambiguous identification of amphetamines analysed. Also, the mass spectra of 4-alkyl-derivatives of 2,5-DMA obtained with CE-MS (Fig. 3) can be useful for future identification in biological matrices as well as in confiscated tablets.

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