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Determination of amphetamines in human whole blood by capillary electrophoresis with photodiode array detection

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Abstract

A capillary electrophoresis (CE) with photodiode array detection (DAD) method for the analysis of amphetamines in human whole blood samples is described. Amphetamines were applied to CE without any derivatization procedure and detected at 200 nm for a rapid and simple analysis. The UV-spectra are show in Fig. 2. Amphetamines were separated within 7 min through an uncoated fused-silica capillary (50 cm × 50 μm ID) using a 100 mM phosphate buffer (pH 2.5). A simple and fast extraction method of amphetamines from human whole blood was developed using acetonitrile. Very clean extracts were obtained in one step. Whole blood drugs free samples were spiked with amphetamine standard solution of known concentration. Linear calibration plots were obtained over a large concentration range, with correlation coefficients higher than 0.998. Recoveries between 81 and 99% were obtained. Limit of detection (LOD) was from 10 to 30 ng ml^{-1} for most of amphetamines, except for MDMA, for which it was 80 ng ml^{-1}.

Keywords: Amphetamines; Human blood; Capillary electrophoresis; Photodiode array

1. Introduction

Amphetamines are powerful stimulants of central nervous system, they increase self-confidence and wakefulness and improve physical performance. The abuse of methamphetamine, amphetamine and their methylendioxy-derivates such as methylenedioxy-amphetamine (MDA), methylenedioxy-methamphetamine (MDMA) and methylenedioxyethyl-amphetamine (MDE) has increased enormously during the last few years. The escalation of abuse of these drugs in Italy is causing serious social problems with an increase of 10–20% a year in Italy [1]. Chronic abuse of amphetamines often leads to hallucinations and psychosis. In response to a growing demand for reliable evidence of amphetamines use, a method providing reproducible results of amphetamines in

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whole blood was developed. Several methods have been developed for this purpose: high-performance liquid chromatography (HPLC) [2,3], gas chromatography–mass spectrometry (GC–MS) [4–9], immunoassays [10–12]. Each method has its own advantages and disadvantages regarding sensitivity, precision and simplicity. Capillary electrophoresis (CE) has been reported as a powerful tool for the simultaneous determination of a number of analytes with high resolution and speed [13,14]. The aim of this study is to design a simple CE–photodiode array detection (DAD) procedure for determination of six amphetamines in whole blood samples. The use of acetonitrile for the extraction is simple, easy to automate, and does not require extraction solvents.

2. Experimental

2.1. Materials and reagents

Methanolic solutions of D-amphetamine, L-methamphetamine, MDA, MDE and MDMA (1 mg ml^{-1}) were purchased from Alltech (State-College, PA), 2,5-Dimethoxy-4-methyl-phenethylamine (PEA) was synthesized in our laboratory. The structure of each analyte is developed in Fig. 1. HPLC-grade methanol was from Carlo Erba (Milan, Italy). Acetonitrile ACS reagent grade was from Riedel de-Haën (Germany). Phosphoric acid RPE-ACS reagent grade was from Carlo-Erba (Milan, Italy). Deionized and distilled water was purified through a Milli-Q system (Millipore).

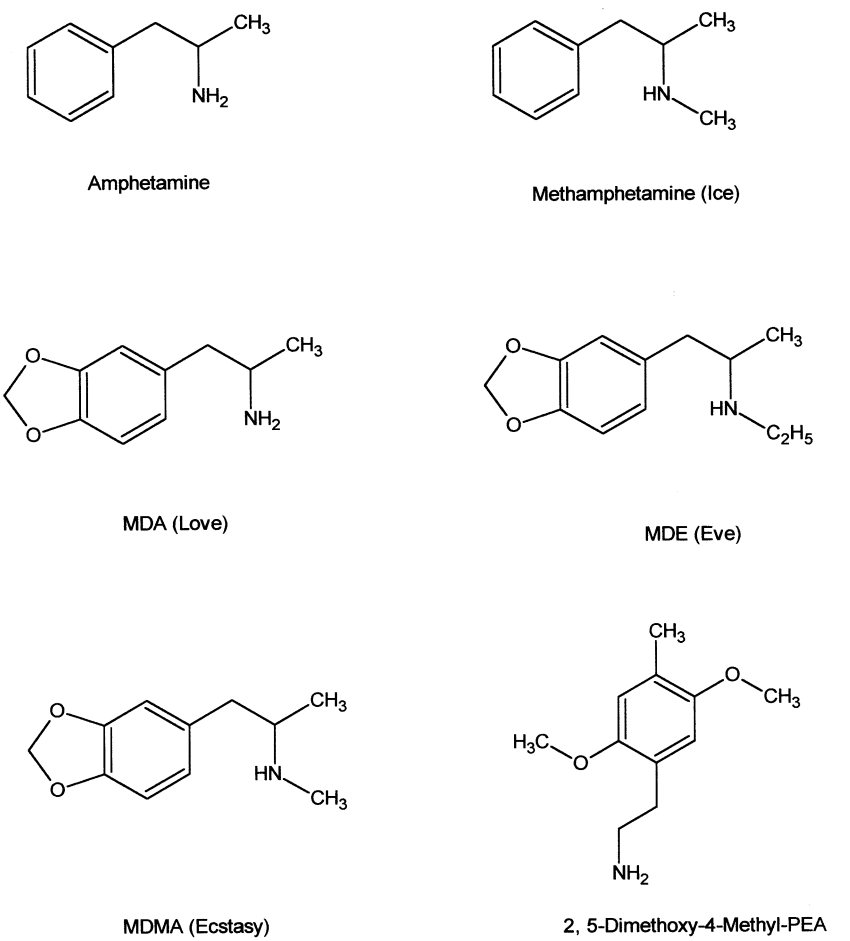

Fig. 1. Chemical structures of the amphetamines studied in this work.

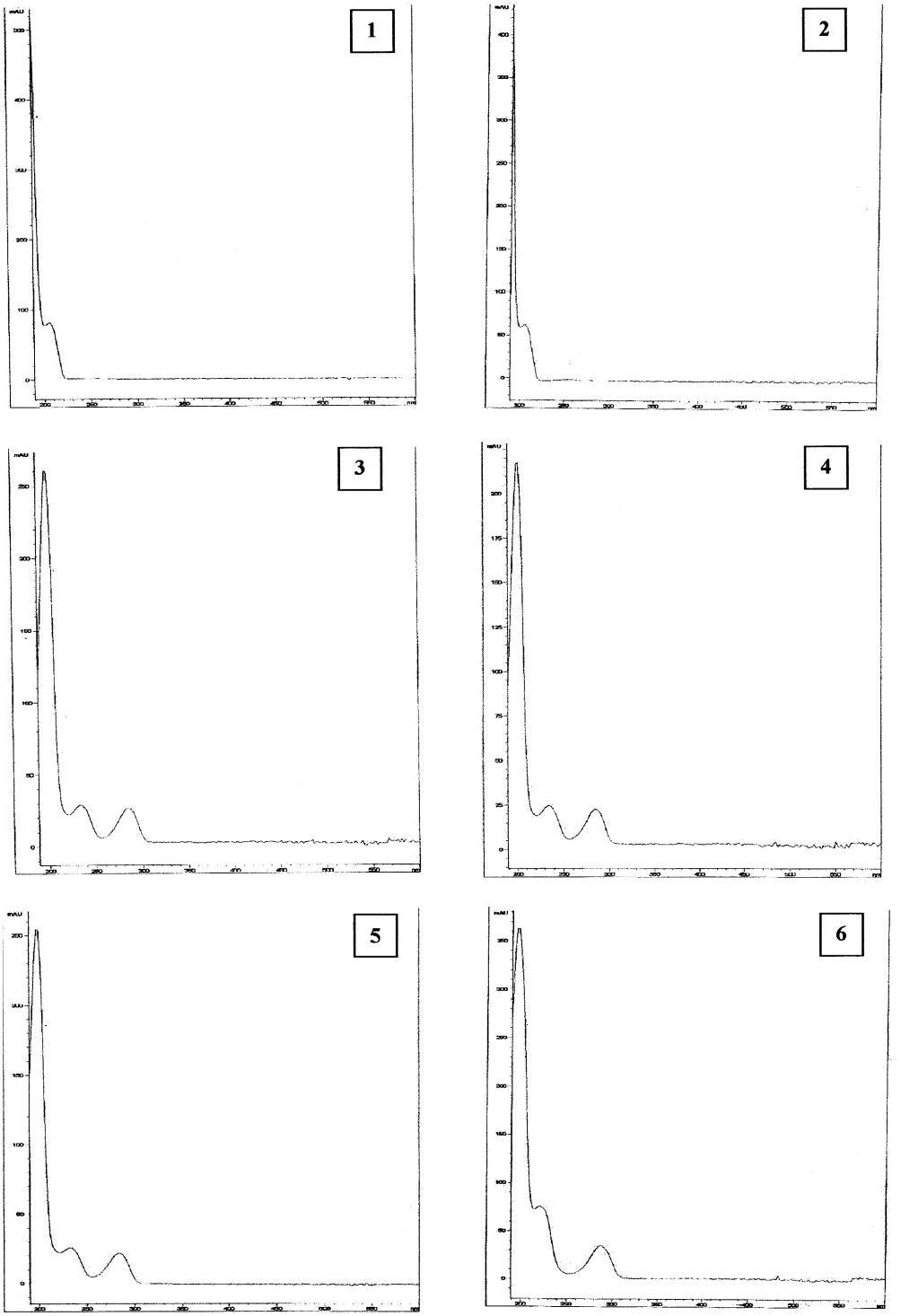

Fig. 2. UV spectra of amphetamines (1, D-amphetamine; 2, methamphetamine; 3, MDA; 4, MDE; 5, MDMA; 6, 2,5-dimethoxy-4-methyl-PEA).

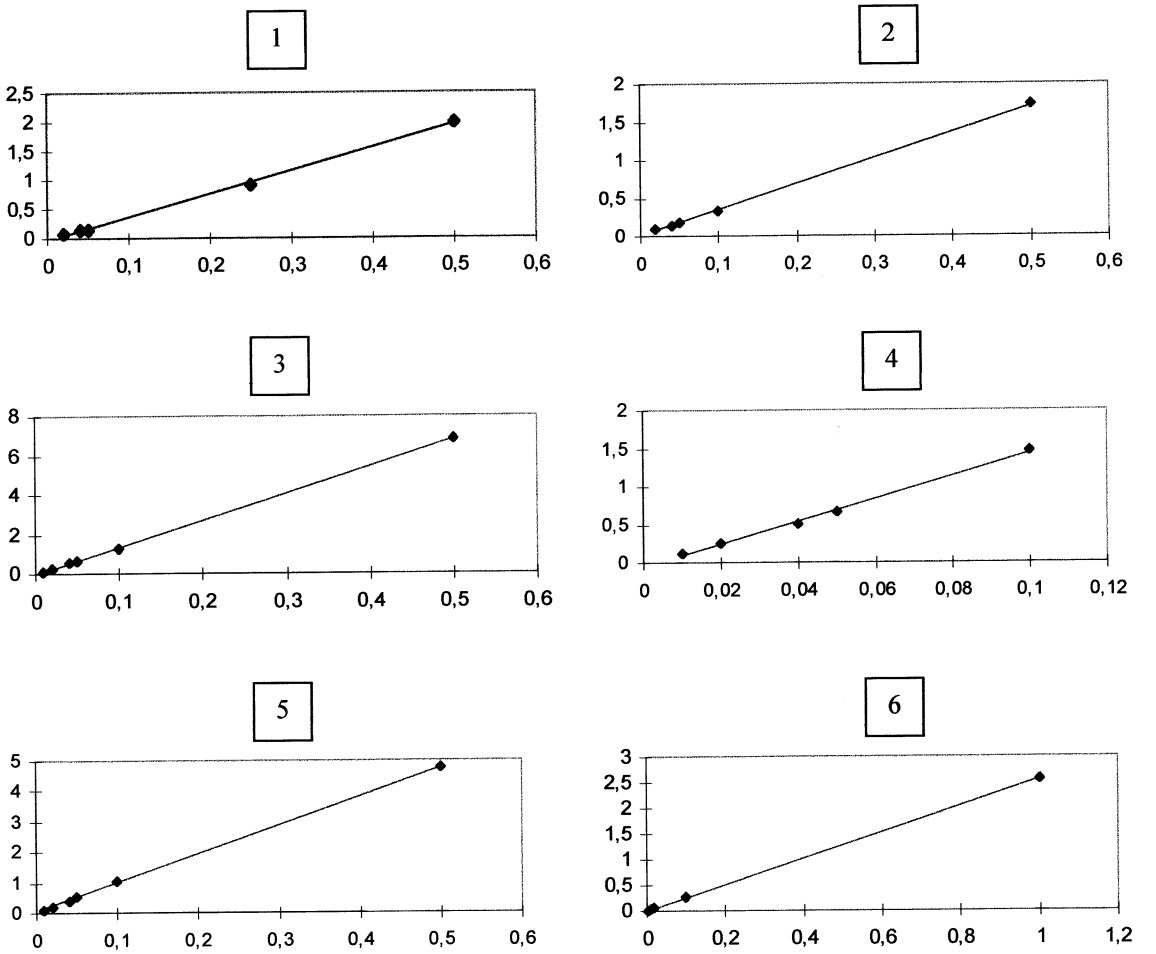

Fig. 3. Calibration curves (1, D-amphetamine; 2, methamphetamine; 3, MDA; 4, MDE; 5, MDMA; 6, 2,5-dimethoxy-4-methyl-PEA).

Milli-Q water was used to prepare all solutions and the buffer. Sodium dihydrogen phosphate 2-hydrate extra pure was supplied by E. Merck Ag. Darmstat (Germany). Drug-free (blank) whole blood was pooled from healthy volunteers at DFCT then stored at −25 °C before use.

2.2. Sample preparation

Primary stock standard solution of amphetamines (500 μg ml^{-1}) was prepared for each compound in methanol–water 1:1 mixture then stored at −25 °C. Stock solutions were diluted with methanol–water 1:1 to produce working standard solutions: 250 , 50, 40 and 20 μg ml^{-1} for D-amphetamine; 100, 50, 40 and 20 μg ml^{-1} for L-methamphetamine; 100, 50, 40, 20 and 10 μg ml^{-1} for MDE, MDA and MDMA; 100, 20, 5 and 2,5 μg ml^{-1} for 2,5-dimethoxy-4-methyl-phenethylamine. Calibration standard samples were prepared spiking the extracts from blank human whole blood samples with the working standard solutions. For recovery determination amphetamines-fortified whole blood was prepared by incubation of 1 ml whole blood with the drugs for 1 h. The whole blood was fortified with 500, 250 and 125 μg of D-amphetamine; 100, 50 and 40 μg of L-methamphetamine, MDE, MDA and

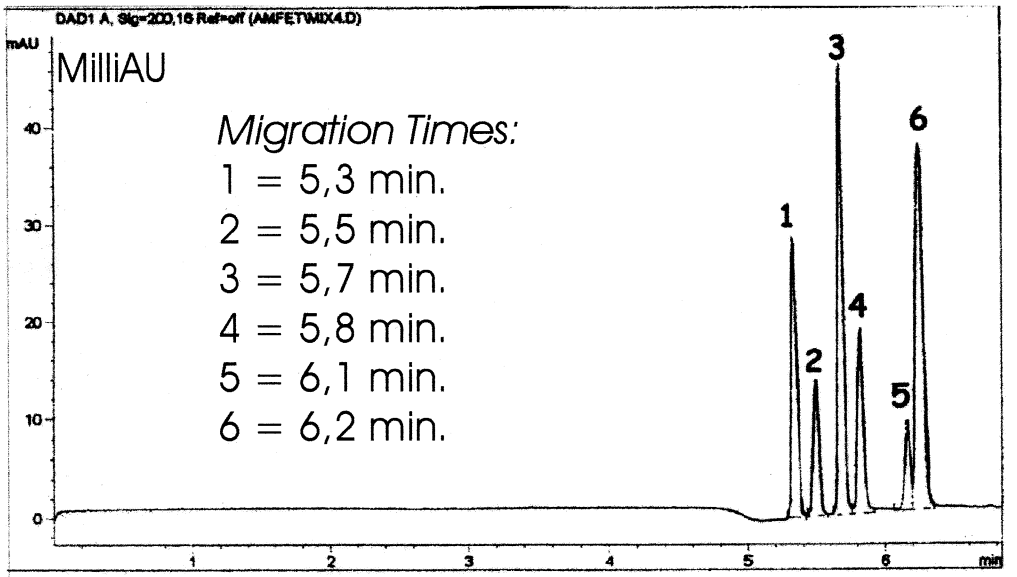

Fig. 4. Electropherogram of amphetamines with DAD detection. CE conditions, capillary, 50 cm × 50 μm ID; detection, 200 nm; temperature, 25 °C; running solution, 100 mM phosphate buffer (pH 2.5); voltage, 10 kV.

MDMA; 800, 230, 125 and 80 μg of 2,5-dimethoxy-4-methyl-phenethylamine.

2.3. Extraction procedure

The assay procedure for whole blood with CE–DAD detection was as follows: to 1 ml of amphetamines-fortified whole blood in a test tube, 20 μl of phosphoric acid dilute (1/5) was added. The mixture was held in an US-apparatus for 30 min and then extracted by addition of 2 ml acetonitrile while slow vortexing. The mixture was shaken vigorously for 1 min on a vortex mixer. The test tube was centrifuged for 10 min at 3000 rpm and the supernatant was decanted into a volumetric flask. The extract was evaporated just to dryness with a stream of nitrogen. Then methanol–water 1:1 mixture (1 ml) was added. This solution was applied to the CE-system.

2.4. Instrumentation and CE conditions

A model HP Capillary Electrophoresis System (Agilent Technologies) was employed for CE–DAD studies. The following conditions were used in all the analyses: pressure injection (10 s with 35 mbar); detection at 200 nm; separation voltage is 10 kV; uncoated fused-silica capillary (50 cm × 50 μm ID effective length 41 cm); temperature 25 °C. The CE–DAD was performed using 100 mM phosphate buffer (pH 2.5) as a running buffer solution.

Table 1
Equations of standard curves and linearity ranges

Compound	y = mAu	Linearity range (μg ml^{-1})
D-amphetamine	$y = -0.042 + 4.005x$	500–20
L-methamphetamine	$y = 0.006 + 3.418x$	500–20
MDA	$y = -0.035 + 13.725x$	500–10
MDE	$y = -0.055 + 14.946x$	100–10
MDMA	$y = -0.038 + 9.417x$	500–10
2,5-Dimethoxy-4-methyl-PEA	$y = 0.005 + 2.557x$	1000–0.25

Table 2
Linear regression with detection limits and quantitation limits obtained by CE–DAD detection

Compound	r	LOD (ng ml^{-1})	LOQ (ng ml^{-1})
D-amphetamine	0.99915	30	200
L-methamphetamine	0.99991	20	70
MDA	0.99991	20	150
MDE	0.99824	30	220
MDMA	0.99991	80	190
2,5-Dimethoxy-4-methyl-PEA	0.99999	10	30

Table 3
Recovery data of spiked human whole blood samples

Compound	Mean recovery (%)	S.D. (%)
D-amphetamine	95	5.74
L-methamphetamine	86	8.14
MDA	98	2.05
MDE	99	2.31
MDMA	84.5	7.00
2,5-Dimethoxy-4-methyl-PEA	81	0.96

3. Results and discussion

A selective extraction of amphetamines based on acidic hydrolysis followed by acetonitrile precipitation of proteins was obtained. The first treatment with phosphoric acid allows the separation between proteins and amphetamines. The following addition of acetonitrile allows the precipitation of proteins. Leaving the active principle in the supernatant. After evaporation of solvent, the residue is solubilized with methanol–water 1:1. This method proved effectiveness for extraction of amphetamines avoiding more treatments with organic solvents and clean-up. The blanks were shown totally wanting of interferences. Furthermore, the experimental procedure is relatively simple. In CE–DAD, separation of amphetamines was examined using 100 mM phosphate buffer at pH 2.5 as running buffer solution [13,14]. The voltage is 10 kV. Clean electropherograms were obtained showing thin, symmetric electropherographic peaks, and all drugs were resolved (Fig. 4). To validate this method, the following parameters were calculated: calibration curve, linear regression, detection limit (LOD), determination limit (LOQ), recovery and repeatability. For each drug, calibration curve was constructed by spiking blank whole blood with known concentrations of amphetamines. Results were reported in nanograms per milliliter for all components. The calibration curves (Fig. 3) were linear in the tested range. Equations of standard curves and linearity ranges are indicated in Table 1. For each amphetamine, effective linear regressions was obtained. The linear regression with detection limits and determination limits obtained by CE–DAD detection are listed in Table 2. The linear regressions were >0.998. The detection limit was from 10 to 30 ng ml^{-1} for most of amphetamines, except for MDMA, for which it was 80 ng ml^{-1}. The LOD and the LOQ were defined as the concentrations obtained calculating the standard deviation of the range multiplied by three and ten times, respectively [15]. Recovery was assessed by extraction and determination of different aliquots of spiked whole blood in the same runs (Table 3). Recovery specimens were prepared by spiking 1 ml of sample with a known concentration of amphetamines. Results are summarized in Table 3 in which are reported the precision data as S.D.%. Comparison studies of several types of blood products indicated that the recovery of the method was not matrix dependent. The samples were extracted and analyzed against a calibration curve. All whole blood was tested for the absence of amphetamines before use. Fig. 5 shows typical electropherograms obtained for human whole blood and that spiked with amphetamines under established conditions. Repeatability run-to-run was evaluated (Table 4). Finally repeatability of the instrument was evaluated calculating the migration time of a standard solution of each amphetamine every ten sample injections (with washing every three injections; Table 5).

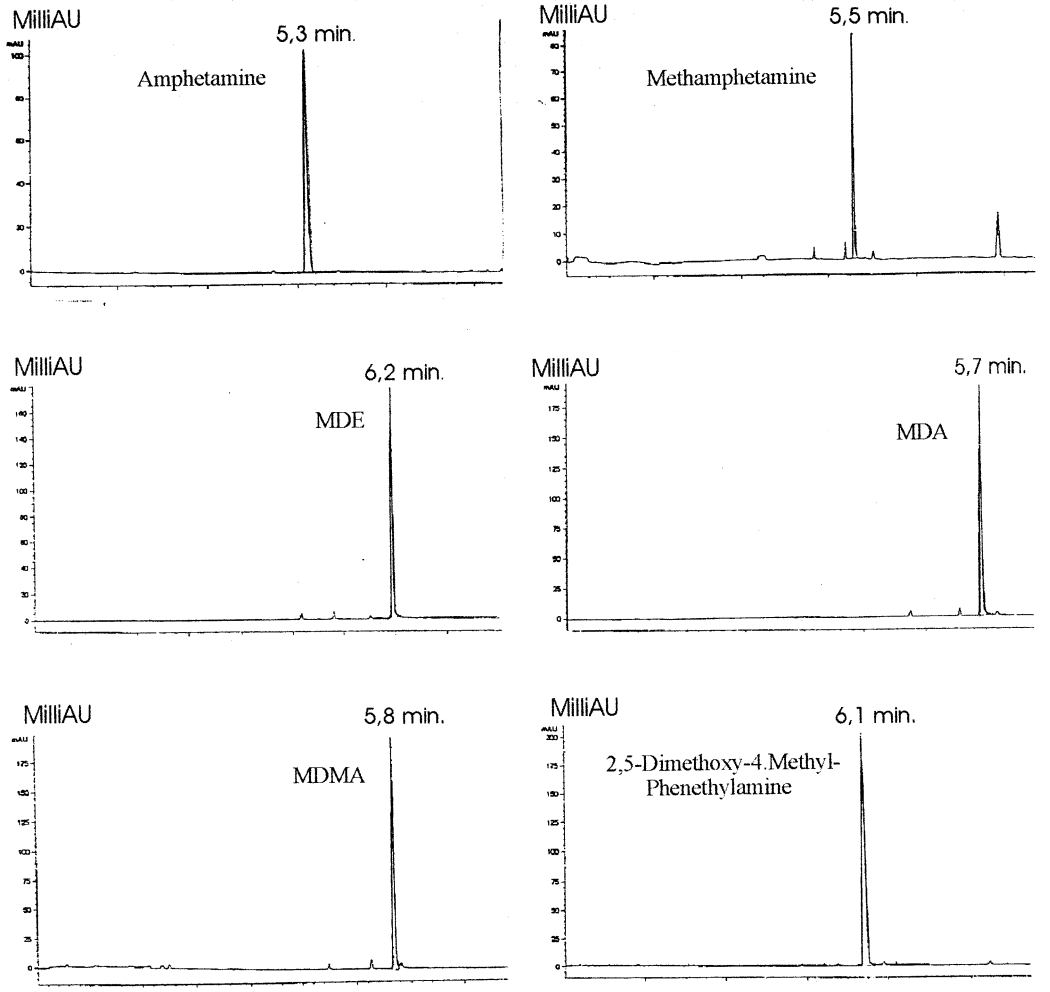

Fig. 5. Electropherogram of six amphetamines with DAD detection (1, D-amphetamine; 2, methamphetamine; 3, MDA; 4, MDMA; 5, 2,5-dimethoxy-4-methyl-PEA; 6, MDE). CE conditions, capillary, 50 cm × 50 μm ID; detection, 200 nm; temperature, 25 °C; running solution, 100 mM phosphate buffer (pH 2.5); voltage, 10 kV.

4. Conclusions

This method was proved effective. The use of acetonitrile for precipitation of proteins allows a selective extraction of amphetamines. It is simple, fast and sensitive. Moreover, the use of dilute phosphoric acid produces almost absolute recovery. Furthermore this method permits a good separation of amphetamines, necessary for 'multi-residuo' analysis. In conclusion, results of the present study show that the proposed acetonitrile-CE method is an efficient and reliable means of

Table 4
Repeatability run-to-run

Compound	S.D.
D-amphetamine	0.00797
L-methamphetamine	0.00760
MDA	0.01468
MDE	0.00809
MDMA	0.02849
2,5-Dimethoxy-4-methyl-PEA	0.4890

Table 5
Repeatability of the instrument

Compound	Retention time	S.D.
D-amphetamine	5.308	0.056
L-methamphetamine	5.487	0.076
MDA	5.670	0.060
MDE	6.240	0.044
MDMA	5.820	0.073
2,5-Dimethoxy-4-methyl-PEA	6.157	0.074

quantitating amphetamines in human whole blood.

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