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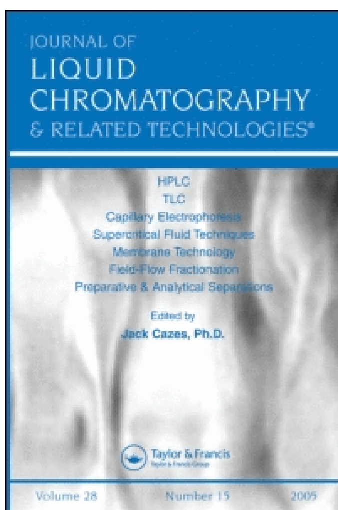

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Determination of p-Methoxyamphetamine by Capillary Electrophoresis with Diode Array Detection from Urine and Plasma Samples

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Abstract: In recent years, a number of newer designer drugs have entered the illicit drug market. The amphetamine derivatives represent the largest group of designer drugs. This paper describes a method for quantifying p-methoxyamphetamine in human plasma and urine by capillary electrophoresis with a diode array detector. Using an aqueous pH 2.5 phosphate buffer, CE analysis gave peaks with good symmetry and reproducible migration time. Under these experimental conditions, the p-methoxyamphetamine was resolved in 7 min and without interferences from biological matrices. The identification using the migration time was confirmed by UV spectra recorded with a diode array detector DAD (190–350 nm). Prior to CE-DAD analysis, a simple solid phase extraction (SPE) was used for sample cleanup. The main advantages of the present method lie in its simplicity and clean and reliable extraction from plasma and urine. The method was validated according to international guidelines.

Keywords: p-Methoxyamphetamine (PMA), Urine, Plasma, Capillary electrophoresis (CE)

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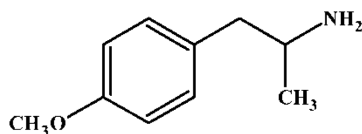
INTRODUCTION

p-Methoxyamphetamine (PMA) is an amphetamine designer drug that has emerged recently on the European illicit drug market. This drug has been associated with a great number of lethal intoxications in Europe,[1,2] United States,[3,4] and Australia.[5]

Monitoring of amphetamines and designer drugs in biological fluids is successfully used for clinical and forensic application and in surveillance of drug substitution. To date, the determination of p-methoxyamphetamine in biological samples has been mainly on gas chromatography coupled with several detection methods, such as mass spectrometry (GC-MS)[3,6] and nitrogen phosphorus detector (NPD).[7] Several other analytical methods are described in literature for screening of p-methoxyamphetamine, such as high performance liquid chromatography with fluorescence detection (HPLC-FL)[8] and immunoassays.[9]

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.[10–12] Beside these chromatographic techniques, the capillary electrophoresis has been established as a powerful method for the toxicological investigation of illicit drugs in biological matrices as well as in confiscated tablets.[13–15]

This paper describes a method for quantification of p-methoxyamphetamine (Figure 1) in biological fluids, by capillary electrophoresis with diode array detection. Using an aqueous pH 2.5 phosphate buffer, CE analysis gave peaks with good symmetry and reproducible migration times. The use of acidic buffers is particularly favourable for the resolution of basic compounds in uncoated fused silica capillaries, since these compounds are present in cationic form.[16] In this study, the PMA was resolved in 7 min and without interferences from biological matrices. The extraction procedure from biological matrices was based on our previous procedure for urine.[17] This method was also successfully applied to plasma extraction. Identification by migration time was confirmed by UV spectra recorded with a diode array detector (190–350 nm). This procedure is simple, clean, and should be easily applied to epidemiological and clinical studies.



p-Methoxyamphetamine (PMA)

Figure 1. Chemical structure of p-methoxyamphetamine.

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EXPERIMENTAL

Reagents and Chemicals

p-Methoxyamphetamine (Figure 1) was synthesised in our laboratory at the maximum level of purity using a slight modification of a method described in literature.[18] 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 carried out using a Perkin-Elmer 1760-X IFT.

The internal standard benzylamine (BZA) was purchased by Merck (Germany). 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. Aqueous stock solution (1.0 mg/mL) of p-methoxyamphetamine was prepared, stored at $+4°C$, and diluted with Milli Q water to appropriate concentrations before use. Internal standard (benzylamine) was added to obtain its 0.5 μg/mL concentration in all cases.

Drug free specimens collected from a healthy adult male were used to make blank and spiked samples containing PMA. The urine and plasma samples were kept frozen at $-20°C$ until analysed.

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Apparatus

Separations in CE were performed using a model HP (Hewlett-Packard) capillary electrophoresis system (Agilent Technologies).

An uncoated fused silica capillary (50 cm $\times$ 50 μm I.D.) was used for the capillary electrophoresis separation. The running buffer consisted of 100 mM sodium phosphate adjusted to pH 2.5 with phosphoric acid. A separation voltage of 10 kV was applied. Samples were injected hydrodynamically with a pressure of 35 mbar for 10 s. The detection was made at 210 nm.

Extraction Procedure from Biological Matrices

A biological 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 appling 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 analyte was 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.

Method Validation

The method was validated according to international guidelines.[19,20] The specificity of the method was evaluated by analysing plasma and urine specimens from 10 healthy non-drug consuming subjects. Linearity was obtained with an average determination coefficient (r^2) >0.99. To construct calibration curves, drug free samples of plasma and urine spiked with PMA at concentrations ranging from 50 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 $y_{LOD} = b + 3SD_b$ and $y_{LOQ} = b + 10SD_b$, where b = intercept and SD_b = standard deviation of intercept.[21]

Repeatibility was evaluated by analysing samples containing 250 ng/mL of p-methoxyamphetamine on the same day in ten replicates (intra-day precision) and over five consecutive day in duplicate (inter-day precision), and by calculating the RSD (relative standard deviation) of the experimentally determined concentrations. Finally, repeatibility of the instrument was evaluated calculating the migration time of a standard solution (250 ng/mL) of p-methoxyamphetamine in ten sample injections (with washing every third injection).

The accuracy was expressed in terms of recovery%. Recovery values were studied by spiking plasma samples at three fortification levels (100–250–500 ng/mL) and analysing six replicates.

RESULTS AND DISCUSSION

Validation was performed with regard to LOD, LOQ, linearity, intra-, and inter-day precisions and accuracies in human plasma and urine. The LOD values for plasma and urine were, respectively, 20.92 ng/mL and 24.26 ng/mL, and the LOQs were 51.40 ng/mL and 59.70 ng/mL. The calibration curves showed linearity in the range of 50–5000 ng/mL for both biological matrices analysed, and the correlation coefficients (r^2) were higher than 0.998. This sensitivity was comparable to that observed by GC-MS for similar amphetamines[3] and was sufficient for confirmatory testing of toxicogical levels of drug consumed.[2,7,22]

Recoveries obtained from spiked plasma and urine were better than 88%. The means of recoveries at three fortification levels were reported in Table 1.

The extraction procedures from plasma and urine allows one to obtain electropherograms free of interfering extraneous peaks (Figure 2). Typical electropherograms extracted from plasma and urine specimens spiked with PMA were shown in Figure 3, where no interference from endogenous substances was observed.

Data for accuracy and precision (Tables 1 and 2) were within required limits.

The CE analysis gave peaks with good symmetry and reproducible migration times. Under these experimental conditions, the PMA was

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Table 1. Results of the recoveries obtained by the standard additions method (n = 5)

Matrices	Spiked level (ng/mL)	Mean value (ng/mL)	Recovery (%)	Mean recovery (%)
Plasma	100	87.3	87.3	88.30
	250	216	86.4	
	500	456	91.2	
Urine	100	91.2	91.2	96.73
	250	250	100	
	500	495	99.0	

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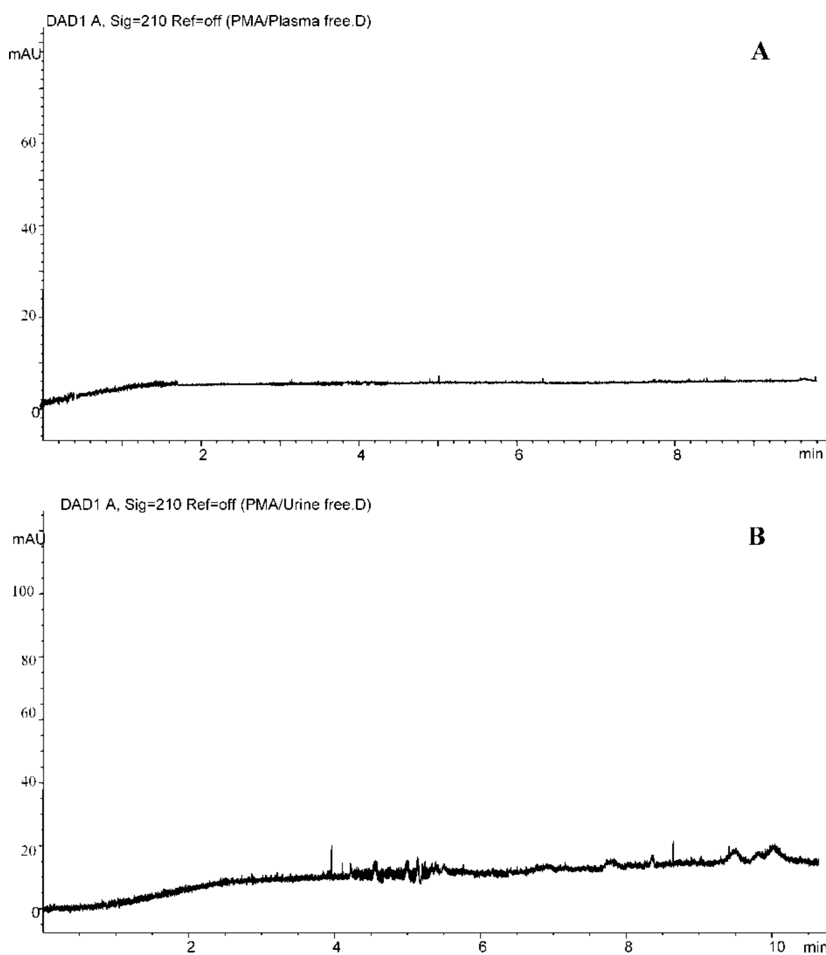

Figure 2. Electropherograms of extracts of plasma blank (A) and urine blank (B).

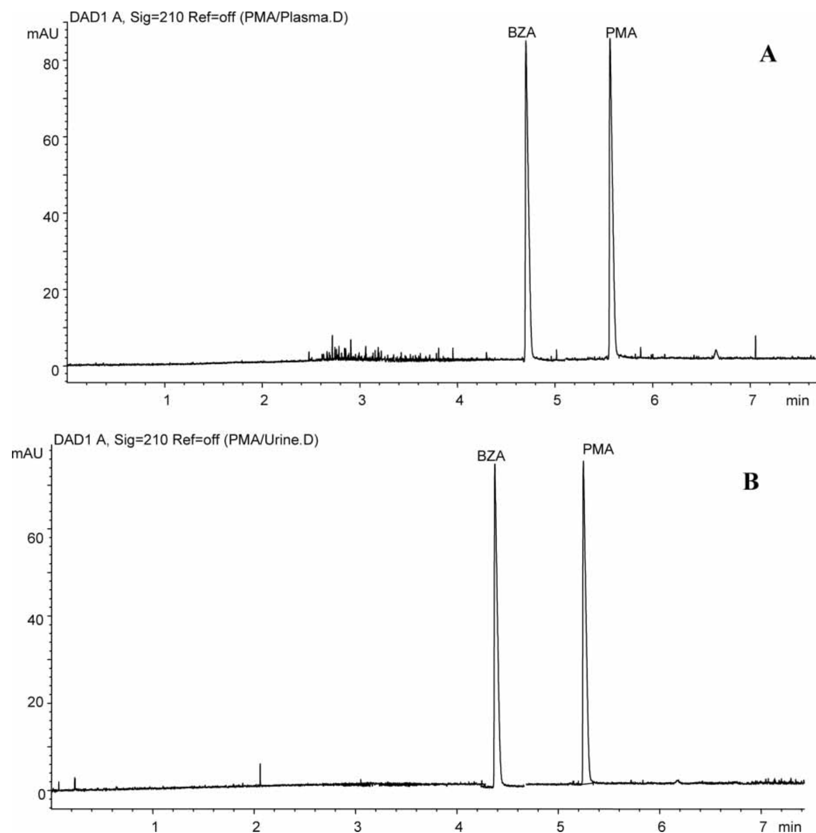

Figure 3. Electropherograms of extracts of plasma (A) and urine (B) spiked with p-methoxyamphetamine (100 ng/mL).

resolved in 7 min and without interferences from matrices. The identification by the migration time was confirmed by UV spectra recorded with a diode array detector (190–350 nm).

This simple extraction procedure should be applicable to routine epidemiological and clinical studies.

Table 2. Intra- and inter-day precision

Compound	Reproducibility intra-day (RSD)	Reproducibility inter-day (RSD)	Repeatability of instrument (RSD)
Plasma	2.67	2.71	0.28
Urine	3.51	2.98	0.78

RSD = relative standard deviation.

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REFERENCES

1. Jacobs, W. Death and p-methoxyamphetamine—an evolving problem. Med. J. Aust. **2002**, *177*, 460.
2. Johansen, S.S.; Hansen, A.C.; Muller, I.B.; Lundemose, J.B.; Franzmann, M.B. Three fatal cases of PMA and PMMA poisoning in Denmark. J. Anal. Toxicol. **2003**, *27*, 253.
3. Kraner, J.C.; McCoy, D.J.; Evans, M.A.; Evans, L.E.; Sweeney, B.J. Fatalities caused by the MDMA-related drug p-methoxyamphetamine (PMA). J. Anal. Toxicol. **2001**, *25*, 645.
4. Ling, L.H.; Marghant, C.; Buckley, N.A.; Prior, M.; Irvine, R.J. Poisoning with the recreational drug p-methoxyamphetamine ("death"). Med. J. Aust. **2001**, *174*, 453.
5. Byard, R.W.; James, R.A.; Gilbert, J.D.; Felgate, P.D. Another PMA-related fatality in Adelaide. Med. J. **1999**, *170*, 139.
6. Peters, F.T.; Schaefer, S.; Staack, R.F.; Kraemer, T.; Maurer, H.H. Screening for and validated quantification of amphetamines and amphetamine- and piperazine-derived designer drugs in human blood plasma by gas chromatography/mass spectrometry. J. Mass Spectrom. **2003**, *38*, 659.
7. Martin, T.L. Three cases of fatal p-methoxyamphetamine overdose. J. Anal. Toxicol. **2001**, *25*, 649.
8. Kaminskas, L.M.; Irvine, R.J.; Callaghan, P.D.; Whitez, M.; Kirkbride, P. The combination of the metaboliti p-hydroxyamphetamine to the central action of p-methoxyamphetamine. Psychopharmacology **2002**, *160*, 155.
9. Lora-Tamayo, C.; Tena, A.; Rodriguez, A. Amphetamine derivate related deaths. Forensic Sci. Int. **1997**, *85*, 149.
10. Mortier, K.A.; Dams, R.; Lambert, W.E.; De Letter, E.A.; Van Calenbergh, S.; Leenheer, A.P. Determination of p-methoxyamphetamine and other amphetamine-related designer drugs by liquid chromatography/sonic spray ionization mass spectrometry. Rapid Commun. Mass Spectrom. **2002**, *16*, 865.
11. Dams, R.; De Letter, E.A.; Mortier, K.A.; Cordonnier, J.A.; Lambert, W.E.; Piette, M.H.; Van Calenbergh, S.; De Leenheer, A.P. Fatality due to combined use of the designer drugs MDMA and PMA: a distribution study. J. Anal. Toxicol. **2003**, *27*, 318.
12. Stanaszek, R.; Piekoszewski, W. Simultaneous determination of eight underivatized amphetamines in hair by high performance liquid chromatography-atmosferic pressure chemical ionization mass spectrometry (HPLC-APCI-MS). J. Anal. Toxicol. **2004**, *28*, 77.
13. Boatto, G.; Faedda, M.V.; Pau, A.; Asproni, B.; Menconi, S.; Cerri, R. Determination of amphetamines in human whole blood by capillary electrophoresis with photodiode array detection. J. Pharm. Biomed. Anal. **2002**, *29*, 1073.
14. Di Pietra, A.M.; Gotti, R.; Del Borrello, E.; Pomponio, R.; Cavrini, V. Analysis of amphetamine and congeners in illicit samples by liquid chromatography and capillary electrophoresis. J. Anal. Toxicol. **2001**, *25*, 99.
15. Nieddu, M.; Boatto, G.; Carta, A.; Sanna, A.; Pisano, M. Simultaneous determination of ten amphetamine designer drugs in human whole blood by capillary electrophoresis with diode array detection. Biomed. Chromatogr. **2005**, *19*, 737.
16. Altria, K. *Analysis of Pharmaceutical by Capillary Electrophoresis*; Chromatographia CE Series, Vieweg: Braunschweig, 1998; Vol. 2.
17. Boatto, G.; Nieddu, M.; Carta, A.; Pau, A.; Palomba, M.; Asproni, B.; Cerri, R. Determination of Amphetamine-derived designer drugs in human urine by SPE

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extraction and capillary electrophoresis with mass spectrometry detection. J. Chromatogr. B **2005**, *814*, 93.
18. Shulgin, A.; Shulgin, A. *Pihkal. A Chemical Love Story*; Transform Press: Berkeley, 1998.
19. Peters, F.T.; Maureer, H.H. Bioanalytical method validation and its implications for forensic and clinical toxicology. Accred. Qual. Assur. **2002**, *7*, 441.
20. Taveniers, I.; de Loose, M.; Van Bockstaele, E. Analytical method validation and quality assurance. Trend Anal. Chem. **2004**, *23*, 533.
21. USP. 23 NF18 Validation of Compendial Methods **1995**, *7* 1983.
22. Becker, J.; Neis, P.; Rohrich, J.; Zorntlein, S. A fatal paramethoxymethamphetamine intoxication. Leg. Med. **2003**, *5*, S138.

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