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SIMULTANEOUS DETERMINATION OF AMPHETAMINE, METHAMPHETAMINE, AND CAFFEINE IN SEIZED TABLETS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

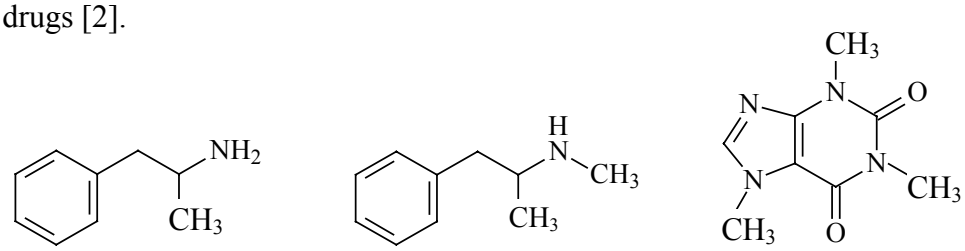
A new, inexpensive HPLC method has been developed for determination of amphetamine and methamphetamine in combination with caffeine, the most widely used adulterant in seized tablets. Reversed-phase chromatography was performed at 40°C with 90:10 (*v*/*v*) aqueous orthophosphoric acid (pH 2.1)–acetonitrile as mobile phase at a flow rate of 1.5 mL min^{-1}. All the analytes were quantified at 205 nm. Statistical validation of the method included determination of selectivity, linearity, accuracy, and precision. The method is rapid, simple, and reproducible and could be used for direct determination of amphetamine, methamphetamine, and caffeine in seized tablets. The identity of each compound in real samples was established by comparing retention times and UV spectra with those of standards.

INTRODUCTION

The amphetamines (uppers, bennies, pep pills) are synthetic stimulants. The original drug is called amphetamine (Fig. 1A) but the group includes dextroamphetamine (dexies), methamphetamine (speed, crystal, meth, crank), and smokeable methamphetamine (ice) (Fig. 1B). These drugs, all of which have similar effects, are available as tablets and capsules that can be taken orally. They are also available as off-white crystals, chunks, and powders which may be sniffed or injected [1].

Methamphetamine is a synthetic stimulant drug used for both medicinal and illicit purposes. Like most stimulants, methamphetamine may induce strong feelings of euphoria and can be addictive. Pure methamphe-

tamine is prescribed by physicians in formulations such as desoxyn. Illicit methamphetamine comes in a variety of forms. Most coveted is a colourless crystalline solid or paste sold on the streets as crystal, crystal meth, clear, glass, shards/shardz, ice, P, or Tina. It is also sold as a less-pure crystalline powder called crank or speed, or in rock formation termed dope, raw, or tweak. It has become one of the world's most widespread illicit drugs [2].



A B C

Fig. 1

The chemical structures of amphetamine (A), methamphetamine (B), and caffeine (C)

Caffeine (Fig. 1C) is a central nervous system stimulant which is used both recreationally and medically to restore mental alertness when unusual weakness or drowsiness occurs. Doses of 100–200 mg result in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination. It also results in restlessness, loss of fine motor control, headaches, and dizziness [3]. It is important to note, however, that caffeine cannot replace sleep, and should be used only occasionally as an alertness aid. While relatively safe for humans, caffeine is substantially more toxic to some other animals, for example dogs, horses, and parrots, because of their much poorer ability to metabolize the compound. Caffeine has a much greater effect on spiders, for example, than most other drugs [4].

Identification and quantification of amphetamine and methamphetamine, with and without caffeine, have been achieved by use of a variety of techniques, for example voltammetry [5], capillary electrophoresis [6,7], infrared spectroscopy [8,9], and liquid chromatography [10–13]. In LC methods chemical derivatization is usually used to make the analytes more amenable to chromatography and/or to enhance sensitivity.

The objective of our study was to develop a new, simple, reversed-phase HPLC method for separation and identification of amphetamine, methamphetamine, and caffeine in seized tablets, without derivatization.

EXPERIMENTAL

Solvents and Reagents

The reagents used were of highest purity (>99.95%). Methanol and acetonitrile of HPLC grade were from Merck (Darmstadt, Germany) and orthophosphoric acid was from Alkaloid (Skopje, R. Macedonia). Authentic samples of amphetamine ($C_9H_{13}N{\cdot}SO_4$), methamphetamine ($C_{10}H_{15}N{\cdot}HCl$), and caffeine ($C_8H_{10}N_4O_2$) were obtained from the United Nations Drug-Control Program (Vienna, Austria).

Sample Preparation

Stock solutions (1.00 mg mL^{-1}) of amphetamine, methamphetamine, and caffeine were prepared in HPLC-grade methanol. The solutions were stored at 4°C until analysis. Series of standards of each of the substances were prepared by progressive dilution of the stock solution. All the samples analyzed (tablets, powders) were seized by Macedonian police in the period from 2005 to 2006, mainly in the area of Skopje. Ground tablets (10 mg) were weighed and dissolved in 7 mL methanol. The solution was sonicated for 5 min, filtered, and diluted to 10 mL with methanol; 20 μL was injected for chromatographic analysis.

Instrumentation and Materials

HPLC was performed with a Varian system equipped with a model 9012 ternary pump and a model 9065 diode-array UV detector. The system was controlled by the software package Varian Star 4.50. Separations were performed on a 250 mm × 4.6 mm, 5-μm particle diameter, LiChrospher 60 RP-select B column protected by a 4 mm × 4.6 mm guard column containing the same packing (both from Merck). Isocratic elution with 90:10 (*v*/*v*) aqueous orthophosphoric acid (pH 2.1)–acetonitrile as optimum mobile phase was performed at 40°C. The flow rate was 1.5 mL min^{-1} and the run time 10 min. Samples were injected through a Rheodyne model 7125 injector valve with 20-μL sample loop. The column was thermostatted with a CH-30 column heater and Eppendorf TC-45 temperature controller.

The identity of each compound was established by comparing retention times and UV spectra from real samples with those obtained from standards. All the analytes were quantified at 205 nm.

RESULTS AND DISCUSSION

HPLC Method, Development and Optimisation

Amphetamine, methamphetamine and caffeine are basic compounds with dissociation constants (pK_a) of 9.9, 10.1, and 10.4, respectively [1–4]. Mobile phase composition and pH (from 2.1 to 4.4), column packing, flow rate, temperature, and detection wavelength were varied and the effect on retention and peak shape were monitored for amphetamine, methamphetamine, and caffeine.

A set of columns of different length and particle size containing C_8, C_{18}, and RP-select B were tested. The final choice of the stationary phase giving satisfactory resolution and run time was the reversed-phase column LiChrospher 60 RP-select B (250 mm × 4.6 mm, 5-μm particle diameter), protected by a suitable guard column. A series of aqueous mobile phases containing buffer solutions of different pH in combination and different volume fractions of acetonitrile and methanol as modifiers were also tested. The best results were obtained by use of 90:10 (*v*/*v*) aqueous orthophosphoric acid (0.01 mol L^{-1}, pH 2.1)–acetonitrile. The mobile phase flow rate was varied from 0.5 to 3.0 mL min^{-1} in steps of 0.5 mL min^{-1}. For simultaneous separation of amphetamine, methamphetamine, and caffeine the optimum flow rate was 1.5 mL min^{-1}. All experiments were performed at 40°C. Elution was monitored over the whole UV range and 205 nm was selected for quantitation because absorption by all the analytes was maximum at this wavelength (Figs 2–4).

Under the optimum chromatographic conditions, the retention times obtained for amphetamine, methamphetamine, and caffeine were 3.94, 4.81, and 5.74 min, respectively. Capacity factors, k, selectivity factors, α, for amphetamine–methamphetamine and for methamphetamine–caffeine, and resolution, R_S for amphetamine–methamphetamine and for methamphetamine–caffeine were calculated from the retention times for amphetamine, methamphetamine, and caffeine and are listed in Table I. The values obtained for these properties ($1 < k < 10$, $\alpha > 1$, $R_S > 2$) show these chromatographic conditions are appropriate for separation and quantification of these compounds. The number of plates is a measure of column efficiency; the values shown in Table I are indicative of good separation efficiency of the column used. The values for the repeatability of the system (*RSD* (%) ≤ 2.0, $n = 7$) show the method is precise.

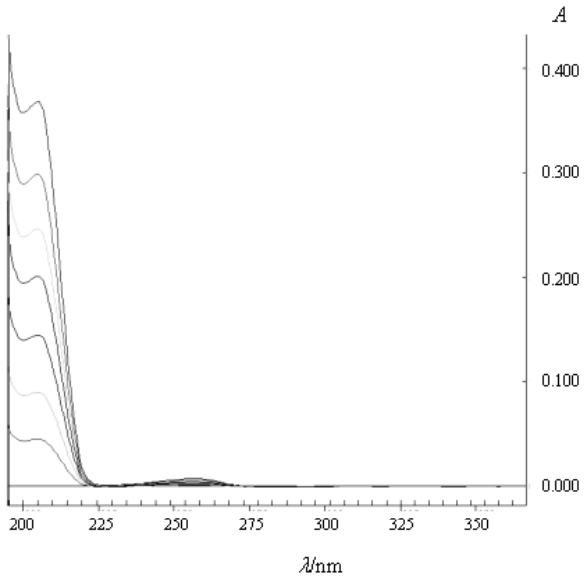


Fig. 2

UV spectra of amphetamine (10–333 μg mL^{-1}) recorded under optimum HPLC conditions

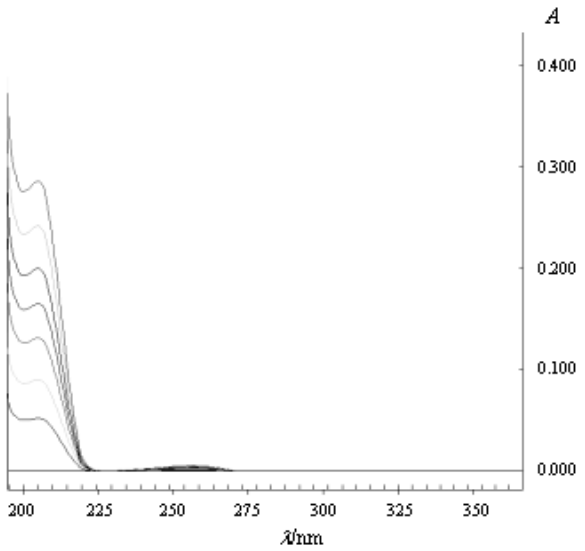


Fig. 3

UV spectra of methamphetamine (10–333 μg mL^{-1}) recorded under optimum HPLC conditions

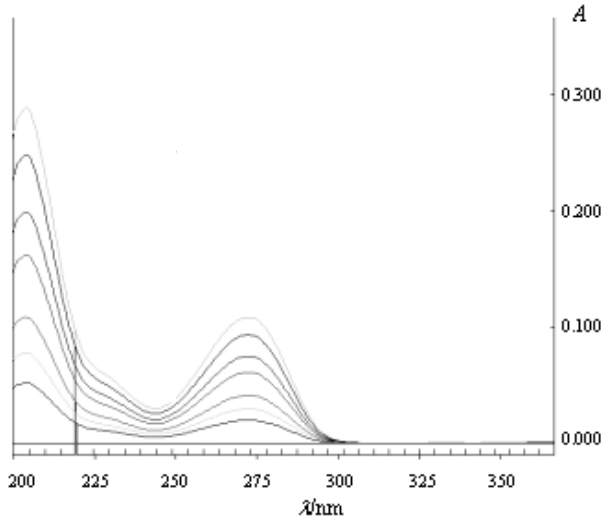


Fig. 4

UV spectra of caffeine (10–333 μg mL^{-1}) recorded under optimum HPLC conditions

Table I

Characteristic performance data obtained for the optimum column

Property	Amphetamine	Methamphetamine	Caffeine
t_R (min)	3.94	4.81	5.74
k	1.28	1.78	3.08
α		1.39	1.73
N	3335	2906	5960
R_S		4.70	4.83
Repeatability (*RSD*, %)	0.45	0.77	0.52

t_0 (migration time, unretained species) = 1.73 min

Validation of the Method

The method was validated for selectivity, linearity, accuracy, and precision.

Selectivity

From the chromatogram shown in Fig. 5B it is evident that under the proposed chromatographic conditions amphetamine, methamphetamine,

and caffeine are completely separated from each other, which indicates the method is selective and can be used for their simultaneous identification and quantification.

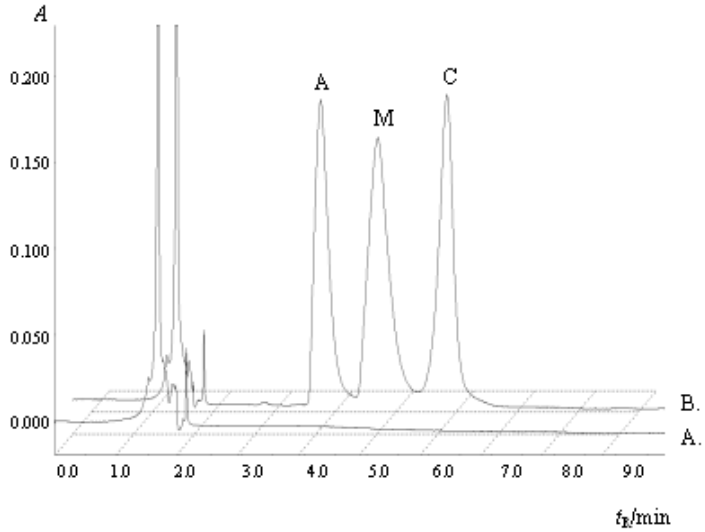


Fig. 5

Chromatograms obtained from methanol (A) and standards (100 μg mL^{-1}) of amphetamine, methamphetamine, and caffeine (B). (A, amphetamine; M, methamphetamine; C, caffeine)

Linearity

The linearity of the method was determined for each component separately, by plotting a calibration graph of peak area against concentration. Least-squares regression of the calibration plots, in the concentration range 5.0 to 333.0 μg mL^{-1} furnished the regression equations:

for amphetamine $y = 4816.1x + 3241$ ($n = 6$, $R^2 = 0.9993$)
for methamphetamine $y = 4588.7x + 2890$ ($n = 6$, $R^2 = 0.9996$)
for caffeine $y = 3027.7x + 241$ ($n = 6$, $R^2 = 0.9996$)

where y is response and x is concentration.

Limits of Detection and Quantification

The limit of detection was calculated as three times the ratio of the *SD* to the slope of the calibration plot in the low-concentration region (i.e. $LOD = 3 \times SD/slope$) and the limit of quantification as ten times this ratio ($LOQ = 10 \times SD/slope$) [14]. By use of the least-squares regression equa-

tions it was found that the limit of detection for amphetamine, methamphetamine, and caffeine was 0.5 μg mL^{-1} and the limit of quantification was 1.5 μg mL^{-1}.

Precision

Intra-day precision was determined by analysis of three series of individually prepared working solutions of amphetamine, methamphetamine, and caffeine at three different concentrations. The results obtained are listed in Table II. The relative standard deviations for all three concentrations were less than 2.63%, illustrating the precision of the method is suitable for routine purposes.

Table II

Results from determination of intra-day and inter-day precision

Nominal concentration (mg mL^{-1})	Intra-day		Inter-day	
	Mean (n = 3) observed concentration (mg mL^{-1})	Relative standard deviation (%)	Mean (n = 15) observed concentration (mg mL^{-1})	Relative standard deviation (%)
Amphetamine				
25	24.63	2.04	24.69	1.72
50	50.30	1.43	50.05	2.10
100	100.30	1.06	99.98	0.99
Methamphetamine				
25	24.76	2.63	24.95	5.11
50	50.10	1.77	50.28	2.27
100	101.43	0.84	100.65	1.92
Caffeine				
25	24.5	1.47	24.77	3.17
50	50.17	2.33	50.32	2.72
100	100.5	0.79	99.36	2.55

Inter-day precision was also determined by analysis of three series of working solutions of different concentration on five different days. The inter-day variation of results throughout the linear range of concentrations is shown in Table II. From these results it is apparent *RSD*s ranged from 0.99 to 2.10% for amphetamine, from 1.92 to 5.11% for methamphetamine, and from 2.55 to 3.11% for caffeine.

These data indicate the method is highly precise and reproducible, both during a single run and during different runs.

Accuracy

Intra-day and inter-day accuracy were determined by analysis of three series of working solutions of the drugs at three different concentrations on five different days. The relative error of the mean observed concentration was calculated as an indication of accuracy. Accuracy data are presented in Table III. The relative errors of −2.32 to 4.80% for amphetamine, −0.72 to 3.70% for methamphetamine, and −3.78 to 2.57% for caffeine indicate the method was sufficiently accurate for analysis of the working solutions.

Table III

Results from determination of intra-day and inter-day accuracy

Nominal concentration (mg mL^{-1})	Intra-day		Inter-day	
	Mean (n = 3) observed concentration (mg mL^{-1})	Relative error	Mean (n = 15) observed concentration (mg mL^{-1})	Relative error
Amphetamine				
25	25.41	1.64	26.2	4.80
50	50.32	0.64	48.84	−2.32
100	101.37	1.37	99.85	−0.15
Methamphetamine				
25	25.51	2.04	25.47	1.88
50	50.78	1.56	51.85	3.70
100	100.74	0.74	99.28	−0.72
Caffeine				
25	24.95	−0.20	24.74	−1.04
50	49.15	−1.70	48.11	−3.78
100	101.24	1.24	102.57	2.57

Determination of Drugs in Seized Tablets

The suitability of this method was assessed by analysis of 145 tablets seized by Macedonian police in 2005 and 2006, mainly in the area of Skopje. Thirty-seven were found to contain amphetamine and caffeine, none contained methamphetamine, and the others contained other drugs. The chromatogram obtained from analysis of a tablet containing 33.5% amphetamine and 52.4% caffeine (colour, yellow–brown; logo ‘cap tag’) is shown in Fig. 6C.

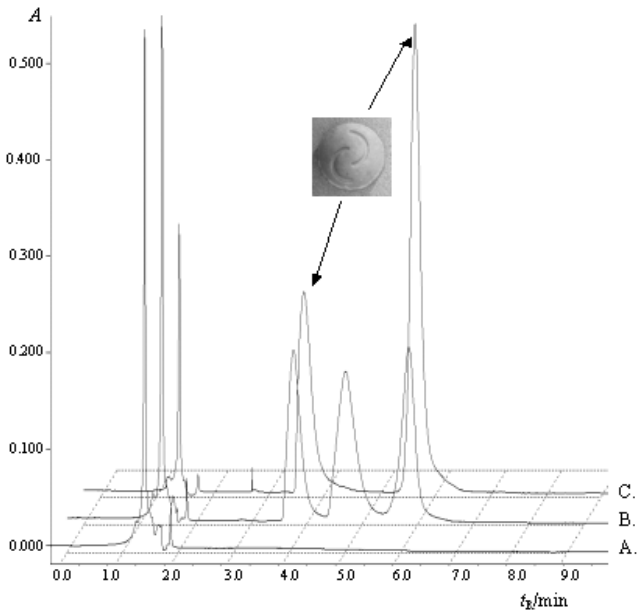


Fig. 6

Chromatograms obtained from methanol (A), standards (75 μg mL^{-1}) of amphetamine, methamphetamine, and caffeine (B), and an extract of a clandestine tablet containing amphetamine and caffeine (C)

CONCLUSION

A new, reversed-phase HPLC method has been developed for simultaneous determination of amphetamine, methamphetamine, and caffeine in seized tablets. The validation data are indicative of good precision and accuracy, and prove the reliability of the method. The method has been used to monitor the amphetamine, methamphetamine, and caffeine content of seized tablets. The results show the method could find practical application in forensic toxicology.

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tamine, methamphetamine, and caffeine standards and real samples of seized tablets.

REFERENCES

[1] http://www.healthunit.org/alcoholdrug/drugs/amphmeth.htm
[2] B.K. Logan, Forensic Sci. Rev., **14**, 133 (2002)
[3] http://mass-spec.chem.edu/VMSL/Caffeine
[4] R. Noever, J. Cronise, and R.A. Relwani, NASA Tech Briefs, **19**, 82 (1995)
[5] N. Velghe and A. Claeys, Analyst, **98**, 372 (1973)
[6] V.C. Trenerry, J. Robertson, and R.J. Wells, J. Chromatogr. A, **708**, 169 (1995)
[7] E. Varesio and J.L. Veuthey, J. Chromatogr. A, **717**, 219 (1995)
[8] N. Sondermann and K.A. Kovar, Forensic Sci. Int., **106**, 147 (1999)
[9] S. Volkanoski, V. Pavlova, and S. Petrovska-Jovanović, Proc. 6th Congress Pure and Applied Chemistry for the Students of Macedonia, O-28, Skopje, Macedonia, 2005
[10] V. Pavlova, B. Mitrevski, V. Sokolovska, and S. Petrovska-Jovanović, Book of Abstracts of 5th Int. Conf. South-East European Chemical Societies and 19th Congr. Chemists and Technologists of Macedonia, Vol. **1**, 77, Ohrid, Macedonia, 2006
[11] C. Cháfer-Pericás, P. Campíns-Falkó, and R. Herráez-Hernández, Anal. Biochem., **333**, 328 (2004)
[12] R. Herráez-Hernández, P. Campíns-Falkó, and J. Verdú-Andrés, J. Biochem. Biophys. Methods, **54**, 147 (2002)
[13] M. Longo, C. Martines, L. Rolandi, and A. Cavallaro, J. Liq. Chromatogr., **17**, 649 (1994)
[14] Varian's Bond Elut Certify Instruction Manual; Varian Sample Preparation Products, Harbor City, CA, USA, 1989