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Rapid determination of amphetamines by high-performance liquid chromatography with UV detection

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Abstract

A liquid chromatographic method with UV detection was developed for the separation and quantification of six amphetamines in the presence of adulterants in illicit drugs. Comparison was made between a regular reversed-phase and a base-deactivated column. The mobile phase composition was optimized by studying the influence of pH, buffer composition and the organic solvent type. Validation was accomplished by examination of the linearity, precision, accuracy, limits of detection and limits of quantification of the method. Amphetamines were quantified in eight tablets obtained from illicit drug seizures and the results were verified by a GC–MS procedure.

Keywords: Forensic analysis; Amphetamines; Amines

1. Introduction

Amphetamine and related compounds are a major class of central nervous system stimulants. Most are classified as controlled or illicit substances in many countries [1,2]. Because of their wide abuse, especially in the case of ecstasy (methylenedioxymethamphetamine, MDMA) and methylenedioxy derivatives, many analytical methods have been developed for their determination. Gas chromatography (GC) coupled with several detection methods [3] (electron-capture, flame ionisation and flame thermionic detectors) is often used for the analysis of different amphetamine derivatives. Furthermore, when coupled to mass spectrometry (MS), GC is considered as the method of choice in forensic laboratories [4–8]. Indeed, GC–MS is a highly sensitive and specific method which can be employed to identify drugs without ambiguity. However, since a derivatization step is necessary with GC methods, artefacts are possible (e.g., false positive results) [7,9,10]. Other analytical methods which are available for the screening and quantification of amphetamines include radioimmunoassays [11], enzyme-multiplied immunoassays [12,13], fluorescence polarization immunoassays [12], competitive binding immunoassays [14], thin-layer chromatography [15] and supercritical fluid chromatography [16]. In addition, reversed-phase liquid chromatography, with or without derivatization, coupled with UV [17,18] or spectrofluorimetric detection [19] has been developed for the identification and quantification of amphetamines. More recently, high-performance capillary electrophoresis (HPCE) has become a complementary analytical tool to the classical GC and HPLC techniques for the analysis of amphet-

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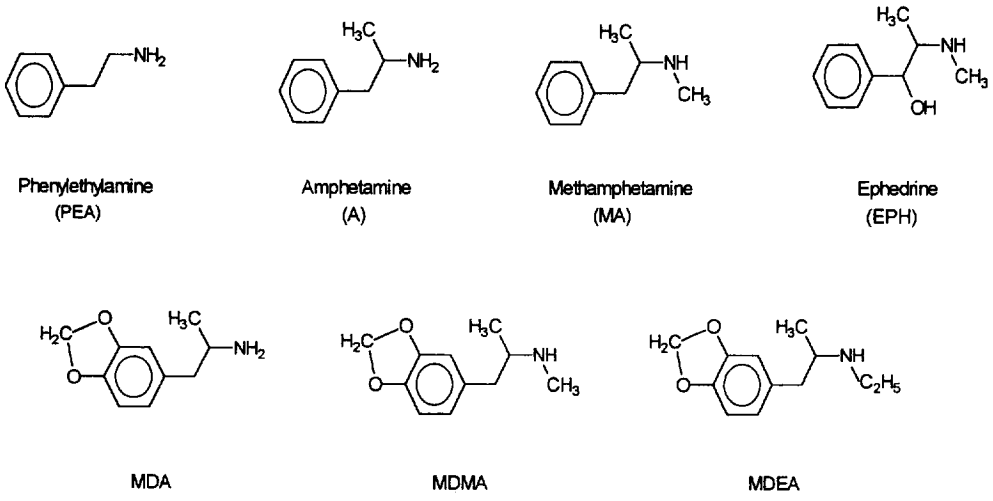

Fig. 1. Structures of analyzed amphetamines.

amines and other illicit drugs [20–22]. Nevertheless, most of these methods have been developed for the separation of only a few amphetamines [6,23–27]. It is therefore desirable to have a complementary method to GC–MS to rapidly determine amphetamines and adulterants in seized tablets.

For this reason, we have developed and validated a method using HPLC–UV for the simultaneous and rapid (<10 min) determination of six amphetamines (Fig. 1) and some adulterants in seized tablets.

2. Experimental

2.1. Chemicals

Standard solutions of 1 mg ml^{-1} of the amphetamines (A, MA, MDA, MDMA, MDEA) and ephedrine (EPH) in methanol were purchased from Alltech (Deerfield, IL, USA). Phenylethylamine (PEA) was obtained from Fluka (Buchs, Switzerland). Seized tablets were kindly supplied by the Forensic Institute of Lausanne (Switzerland). Acetonitrile was purchased from Maechler (Basel, Switzerland). Ultrapure water was supplied by a Milli-Q RG unit from Millipore (Bedford, MA, USA). All other reagents, solvents and substances were analytical-grade reagents from Fluka.

2.2. Equipment and chromatography

Experiments were carried out on a Gilson HPLC system (Gilson Medical Electronics, Villiers-le-Bel, France), equipped with an ASTED-XL autosampler injector and an oven. The Gilson 712 HPLC software was used for instrument control, data acquisition and data analysis. Detection was carried out using a Hewlet-Packard 1050 Series variable wavelength UV–visible detector (Palo Alto, CA, USA) set at 200 nm (8 nm bandwidth).

The samples were also analyzed with a Hewlett-Packard (HP) 5890 gas chromatograph fitted with a HP 7673A automatic liquid sampler and equipped with a HP 5971 mass selective detector. The analytes were separated with a HP Ultra-2 capillary column (fused-silica coated with 5% phenylmethylsilicone phase, 0.33 μm film thickness, 25 m×0.2 mm I.D.). Helium was used as the carrier gas and the column head pressure was set at 15 p.s.i. (1 p.s.i.=6894.76 Pa). 1 μl of the samples was injected using the splitless mode. The injection port and the transfer line were set at 180°C and 280°C, respectively. The electron multiplier was set at 150 V above the tune value. Full-scan mass spectra were collected between 40 and 410 amu at 1 scan s^{-1}. The GC oven temperature was initially held at 70°C for 1 min and then heated to 300°C at a 10°C min^{-1}. Data acquisition and analysis were performed with a HP 7958 B

Chemstation using a Pascal operating software. Data was automatically processed with macros using the Pfleger, Maurer and Weber database for reference mass spectra and unequivocal identification.

2.3. Columns and mobile phases

Two columns were used for the separation of amphetamines: a RP-18 Nucleosil 100, 5 μm and a RP-18-AB Nucleosil 100, 5 μm (Macherey–Nagel, Oensingen, Switzerland) 125×4 mm I.D. Both columns were thermostated to 40°C. Mobile phases were composed of phosphate or acetate buffer (depending on the pH, pH adjustment with 1 *M* HCl or 1 *M* NaOH) mixed with variable proportions of acetonitrile or methanol. The separation was conducted in isocratic elution mode at a flow-rate of 1 ml min^{-1}. Injection volume was 20 μl.

2.4. Sample preparation

The calibration and external standard solutions of EPH, A, MA, MDA, MDMA and MDEA were prepared by dilutions of standard 1 mg ml^{-1} methanolic solutions of each amphetamine in 0.1 *M* HCl.

Before analysis, each tablet was pulverized with a mortar and a pestle to a fine, homogeneous powder. Stock solutions were prepared by dissolving 10–20 mg of each tablet in 10 ml of 0.1 *M* HCl (this procedure was repeated twice for each tablet). Solutions were sonicated for 30 min to increase solubilities, then vortex-mixed and filtered through 0.45 m nylon Titan syringe filters (Scientific Resources, Eatontown, NJ, USA). An appropriate dilution of the filtered solutions was injected in duplicate.

2.5. Selectivity

The selectivity of the method was tested by injecting 10 μg ml^{-1} solutions of each amphetamine separately. Possible interferences of several possible adulterants were also tested by injecting 10 μg ml^{-1} solutions of phenylethylamine (PEA), caffeine, acetylsalicylic acid (AAS), paracetamol, saccharose, lactose, mannitol and sodium chloride.

2.6. Data analysis

Calibration curves were carried out for each amphetamine at concentrations between 0.50 and 20.0 μg ml^{-1} (0.50, 2.0, 5.0, 10.0, 15.0 and 20.0 μg ml^{-1}). Experimental values were plotted as a function of theoretical values. Standard calibration curves were obtained from unweighted least-squares linear regression analysis. The linearity of the method was statistically tested. Confidence intervals were calculated for the intercept and the slope (Student *t*-test, 95% confidence level). Six duplicate determinations of each compound at two concentration levels (0.5 and 10.0 μg ml^{-1}) were performed to calculate precision within the same day (repeatability or run-to-run reproducibility). In addition, this procedure was repeated on three different days to test the day-to-day reproducibilities. Average values of six sample replicates (10.0 μg ml^{-1}) of each amphetamine, injected in duplicate were used to calculate the accuracy of the methods. Accuracies were calculated as the ratio of experimental to theoretical values and were expressed as percent recovery.

Detection limits (LOD) and quantification limits (LOQ) were expressed as mg g^{-1} of amphetamines in a tablet and based on a signal-to-noise ratio of 3:1 and 10:1, respectively.

3. Results and discussion

3.1. Choice of the stationary phase

Amphetamines are basic compounds with pK_a values of about 10 and thus are cationic species for mobile phases buffered at a pH lower than 8. They can be retained by two mechanisms on reversed-phase silica material: either Van der Waals interactions with hydrocarbon moiety of octadecyl chains or by an ion-exchange interaction with the residual silanol groups of the silica. In order to obtain good chromatographic performance (i.e., high efficiency, low asymmetry factors and good resolution), this second interaction has to be eliminated or reduced. To that effect, several base-deactivated silica materials have recently been developed [28] which avoid the use of blocking agents such as triethylamine (TEA) [29,30] in the mobile phase.

Results obtained for the separations of the model compounds EPH, A and MDA with a conventional RP-18 and a base-deactivated RP-18 stationary phase are shown in Fig. 2. Capacity factors (k'), number of theoretical plates (N) and asymmetry factors (A_s) are reported as a function of the pH of the mobile phase. In all cases, the base-deactivated phase (RP-18-AB) gave higher efficiency and lower asymmetry and capacity factors which allowed a rapid separation of the amphetamines with a good resolution as shown in Fig. 2.

Moreover, a strong influence of the residual silanol groups was observed for the separation of amphetamines on the conventional reversed-phased silica in comparison with the base-deactivated silica. Asymmetry factors vary only slightly as a function of pH for the RP-18-AB, whereas a large drop in A_s is observed for the RP-18 column as the pH decreases. Curves for the A_s, N and k' values of the RP-18 material all have a sigmoidal shape resembling an acid–base titration curve with an inflexion point of about 4.2. This value is in good agreement with the published pK_a values of silanol functional groups [28]. It would appear that for pH values below 4, amphetamines are retained only by Van der Waals interactions (gaussian peaks), whereas at pH> 4.0, deprotonated acidic silanol groups interact strongly with amphetamines (peak tailing). This phenomenon is highly reduced with a base-deactivated support since the free silanol groups are almost completely deactivated.

3.2. Mobile phase composition

Several mixtures of acetonitrile or methanol, with acetate or phosphate buffer solutions were tested for the separation of amphetamines. At all pH values tested, no separation was obtained between MDA and MA with methanol as the organic solvent despite concentrations as low as 3%. Only acetonitrile gave a good resolution of the six amphetamines (Fig. 3): log k' values decreased as a function of the percentage of acetonitrile in the solvent. Chromatographic conditions were best for acetonitrile concentrations of 7–10% with an optimum at 9% where all peaks were gaussian (A_s<1.30) and well-resolved (R_s>1.4).

Phosphate and acetate solutions are generally used to buffer the mobile phases: phosphate is employed for solutions in the pH range of 2.0–3.5 or 6.0–8.0 (pK_{a_1}=2.15, pK_{a_2}=7.10) and acetate for solutions in the pH range of 3.5–6.0 (pK_a=4.75). No amphetamine peaks were detected when acetate buffer was used because this latter absorbs appreciably at wavelengths below 220 nm. This phenomenon limits the usefulness of the acetate buffer for absorbance detection at 200 nm. Phosphate solutions were thus employed after adjustment to the desired pH value. As shown in Fig. 2, good chromatographic performance was obtained at pH values below 4.2. The optimal pH range was between 3.4 and 3.8 which ensured good resolution of all tested amphetamines and adulterants, especially the acetylsalicylic acid and the MDEA. Although ionic strength had no influence on amphetamine separation at these pH, the value was nonetheless fixed to 20 mM to obtain complete resolution of the adulterants.

3.3. Selectivity

Chromatograms of amphetamines and some interfering compounds are shown in Fig. 4. PEA, AAS, paracetamol and caffeine were separated whereas the sugars and salts were not detected by UV detection nor did they interfere. Thus, the method proved to be selective.

3.4. Data analysis

The linearity of the method for each amphetamine was tested in the range of 0.5 to 20 μg ml^{-1}. Correlation coefficients (r) obtained from the plot of experimental values as a function of theoretical values were always greater than 0.995. The intercepts and the slopes were not significantly different from 0.00 and 1.00 respectively (Student t-test, $P<$ 0.05). Therefore, for all amphetamines, the method gave a linear response without systematic errors (fixed or relative).

Repeatabilities and reproducibilities were calculated as relative standard deviations (R.S.D.): repeatabilities were in the range of 2.3–8.9% (500 ng ml^{-1}) and 0.7–3.2% (10 mg ml^{-1}) and reproducibilities were in the range of 4.0–15.0% (500 ng

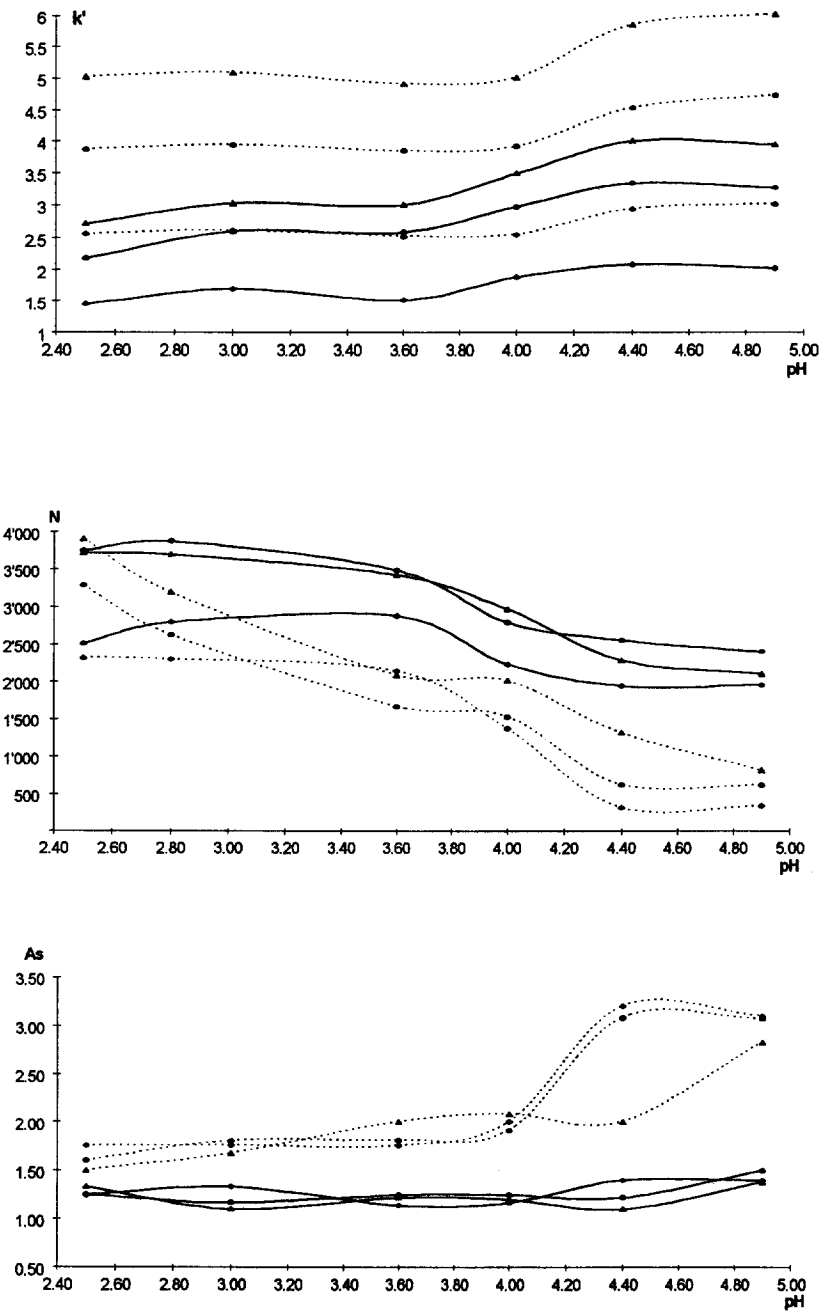

Fig. 2. Comparison of the influence of pH on the capacity factors (k'), the efficiency (N) and the asymmetry factor (A_s) of amphetamines on a C_{18} regular (RP-18) and a C_{18} base-deactivated (RP-18-AB) column for EPH, A and MDA: EPH-RP18 (♦- - -♦); EPH-RP18AB (♦—♦); A-RP18 (●- - -●); A-RP18AB (●—●); MDA-RP18 (▲- - -▲); MDA-RP18AB (▲—▲).

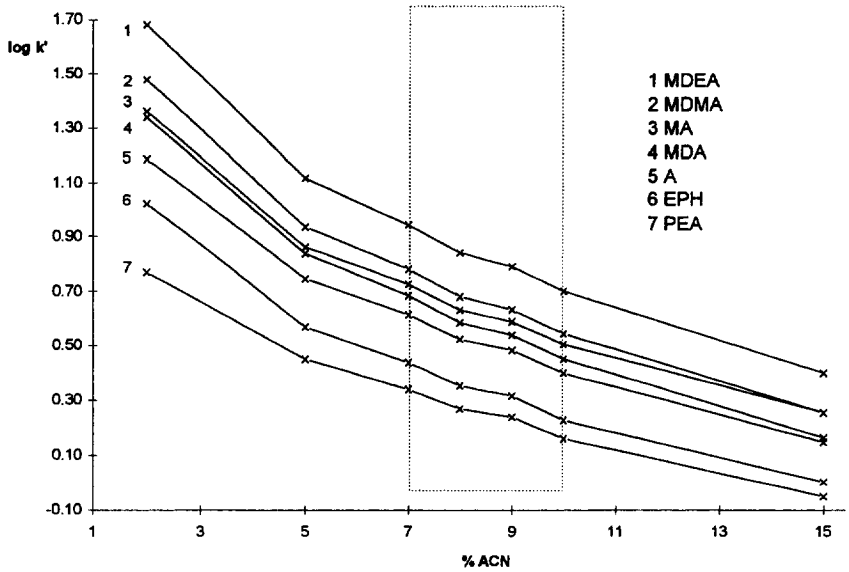

Fig. 3. Log k' as a function of the percentage of acetonitrile in the mobile phase for six amphetamines on a RP-18-AB phase (other conditions see text).

ml^{-1}) and 1.5–6.0% (10 mg ml^{-1}) for six replicate determinations. Furthermore, accuracy of the experimental results was always within 98.7–100.9% of the theoretical values. LOD and the LOQ values showed a good sensitivity for the determination of the six tested amphetamines (Table 1).

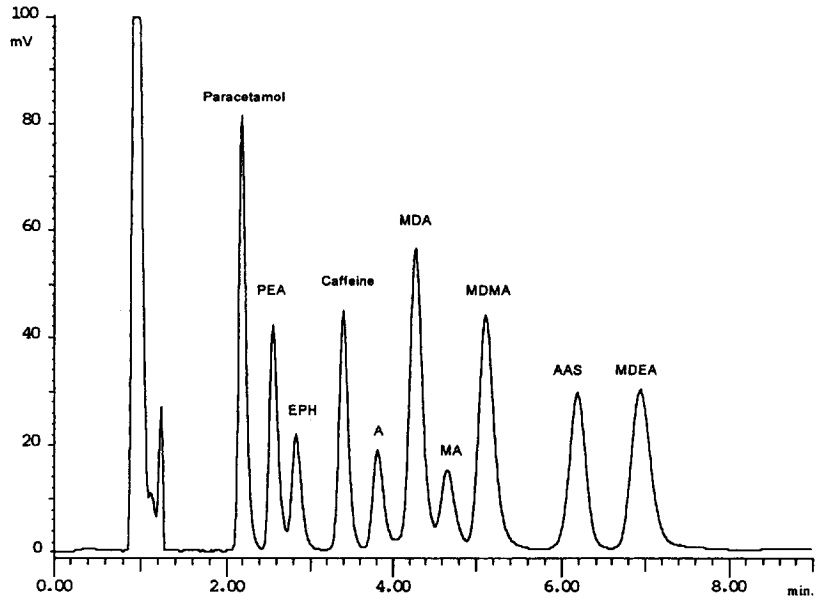

Fig. 4. Separation of six amphetamines and some adulterants by HPLC on a Macherey–Nagel column (125×4 mm) C_{18} AB Nucleosil 100 (5 μm), mobile phase: 20 mM NaH_2PO_4 solution adjusted at pH 3.8–acetonitrile (91:9), flow-rate: 1 ml min^{-1}, UV detection set at 200 nm.

Table 1
Data analysis of the method for the six tested amphetamines

Compound	Linear regression equation $y=ax+b$ ($n=12$)		LOD (mg g^{-1})	LOQ (mg g^{-1})	Repeatability R.S.D. (%)		Reproducibility R.S.D. (%)		Accuracy (%)
	Slope, b (S.D.)	Intercept, a (S.D.)			500 ng ml^{-1}	10 mg ml^{-1}	500 ng ml^{-1}	10 mg ml^{-1}	
EPH	1.036 (0.06)	−0.194 (0.62)	$1.0\ 10^{-4}$	$3.3\ 10^{-4}$	2.3	1.9	15.0	2.8	100.0
A	1.053 (0.09)	−0.034 (0.89)	$9.0\ 10^{-5}$	$3.0\ 10^{-4}$	5.3	3.2	13.9	4.5	98.8
MA	1.059 (0.09)	−0.248 (0.73)	$1.1\ 10^{-4}$	$3.7\ 10^{-4}$	8.9	1.9	14.6	2.3	99.2
MDA	1.007 (0.08)	0.009 (0.76)	$4.0\ 10^{-5}$	$1.3\ 10^{-4}$	5.0	1.7	7.6	1.5	98.9
MDMA	1.030 (0.46)	−0.112 (0.59)	$5.0\ 10^{-5}$	$1.7\ 10^{-4}$	4.2	1.2	7.2	1.5	98.7
MDEA	1.024 (0.46)	−0.136 (0.58)	$6.0\ 10^{-5}$	$2.0\ 10^{-4}$	2.6	0.7	8.5	2.0	99.5

3.5. Application to illicit tablets

Eight tablets seized by the Swiss Police authorities were analysed quantitatively by HPLC–UV. We observed that only one tablet contained MDMA (402 mg g^{-1}). All other tablets were composed of MDEA (263–434 mg g^{-1}). The same tablets were analysed qualitatively by GC–MS (data not shown). Both GC and HPLC methods gave the same composition (amphetamine-like compounds).

4. Conclusion

The appropriateness of using a base-deactivated column in comparison to a regular reversed-phase column was demonstrated for the separation of amphetamines without the need of a blocking agent in the mobile phase. The composition of the mobile phase was optimized to enable the separation of six amphetamines and several adulterants in less than 10 min. Validation of the method showed a good linearity in the range of 0.5–20.0 μg ml^{-1} for all tested amphetamines. Moreover, the method was precise and accurate and no interferences were observed with adulterants. The method was applied to seized tablets and qualitative results were confirmed by GC–MS analysis.

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