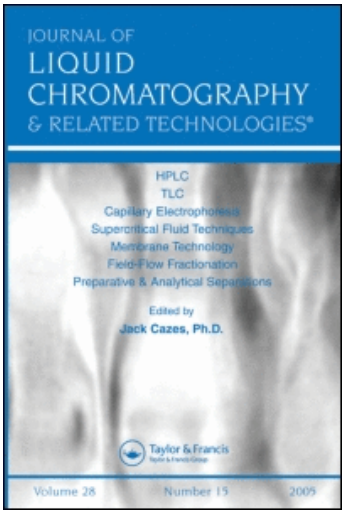


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# Simple and Fast Determination of Some Phenethylamines in Illicit Tablets by Base-Deactivated Reversed Phase HPLC

Marco Longo[a]; Cristina Martines[a]; Laura Rolandi[b]; Aldo Cavallaro[a]
[a] Presidio Multizonale di Igiene e Prevenzione, Milano, Italy [b] Polidinico S. Matteo Piazzale Golgi, Pavia, Italy

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# SIMPLE AND FAST DETERMINATION OF SOME PHENETHYLAMINES IN ILLICIT TABLETS BY BASE-DEACTIVATED REVERSED PHASE HPLC

**MARCO LONGO[1]*, CRISTINA MARTINES[1], LAURA ROLANDI[2], AND ALDO CAVALLARO[1]**

[1]*Presidio Multizonale di Igiene e Prevenzione*
*Via Juvara, 22 - 20129 Milano - Italy*
[2]*Policlinico S. Matteo*
*Piazzale Golgi, 2 - 27100 Pavia - Italy*



ABSTRACT

A high performance liquid chromatographic (HPLC) method for the simultaneous identification and quantitation of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), N-methyl-3,4-methylenedioxyamphetamine (MDMA), and N-ethyl-3,4-methylenedioxyamphetamine (MDE) in presence of caffeine and ephedrine in illicit tablets is described. A simple and rapid sample preparation procedure was applied in order to allow a high number of samples to be processed per day. The chromatographic separation was performed on a commercially available base-deactivated octadecyl silica column with a gradient system using acetonitrile and 20 mM monobasic potassium phosphate buffer. The flow rate was 1.5 ml/min and peak detection was performed at 220 and 280 nm. Peak identity and homogeneity were determined by mapping of peaks in the 195-370 nm range. Quantitative analysis was performed using external standard method in the concentration range studied. Linearity was evaluated in the range 10-500 µg/ml for the 5 substances in exam and for ephedrine, and in the range 3-100 µg/ml for caffeine. Correlation coefficients ranged from 0.9985 to 0.9999. Good accuracy and between-day precision were achieved.

## INTRODUCTION

Amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), N-methyl-3,4-methylenedioxyamphetamine (MDMA), and N-ethyl-3,4-methylenedioxyamphetamine (MDE) are drugs of abuse frequently encountered in tablets of illicit provenience.

Psycho-stimulating effect of amphetamines is well known. MDA and its N-alkyl derivatives are reported to act primarily as central nervous system stimulants that may be hallucinogenic in large doses.(1)

Several papers refer to the high performance liquid chromatographic analysis of amphetamines (2-6) and MDAs (6-8) but no method is simple enough to be employed for the routine simultaneous identification and quantitation of the drugs mentioned above.

A major problem associated with the analysis of basic drugs by reversed phase-high performance liquid chromatography (RP-HPLC) is peak tailing caused by ionic interactions between protonated drugs and free silanol groups of the packing material. These interactions may be reduced by using a very acidic mobile phase, modifying ionic strength of the buffer or adding basic modifiers such as tertiary amines.

A second approach, whose application in analytical toxicology has been recently investigated (9,10), is the use of base-deactivated columns in which free silanol groups are masked through various (often not specified) procedures. Analysis performed on such columns show satisfactory peak shape at higher pH values than those required on conventional RP columns. This results in increased column stability and life span.

The utility of photo diode array detection (DAD) in the analysis of illicit drugs has been shown in a number of papers

(6,11-15). Evaluation of peak purity and variation of wavelength during the analysis are two important features of the DAD.

In this paper we describe a simple and fast routine method for the simultaneous identification and quantitation of amphetamine, methamphetamine, MDA, MDMA, and MDE in illicit tablets in presence of ephedrine and caffeine (two common adulterants normally found in "street" samples).

## EXPERIMENTAL

### Reagents and Chemicals

Ephedrine, amphetamine, methamphetamine, and caffeine were obtained from Sigma Chemical Company (St. Louis, MO, USA). MDA, MDMA, and MDE were obtained from Alltech (Deersfield, IL, USA).

HPLC grade acetonitrile was provided by Carlo Erba (Milan, Italy). Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

All other chemicals were analytical grade.

### Apparatus

The HPLC system consisted of a Model LC 410 quaternary pump equipped with a Model LC 235 diode array detector, a Model ISS 200 autosampler, a Model 1020D integration system, and a Model GP100 printer-plotter (Perkin Elmer, Norwalk, CT, USA).

### Liquid chromatographic conditions

The separation was performed on a 15 cm x 4.6 mm I.D. 5 µm particle size Suplex pKb100 column with a Supelguard precolumn

**TABLE 1**

Concentration Ranges and Correlation Data for the Compounds examined

| COMPOUND | CONCENTRATION RANGES (µg/ml) | CORRELATION COEFFICIENTS (r) |
|---|---|---|
| Ephedrine | 10-500 | 0.9985 |
| Amphetamine | 10-500 | 0.9994 |
| Methamphetamine | 10-500 | 0.9996 |
| MDA | 10-500 | 0.9997 |
| MDMA | 10-500 | 0.9997 |
| MDE | 10-500 | 0.9999 |
| Caffeine | 3-100 | 0.9999 |



containing the same stationary phase (Supelco Inc., Bellefonte, PA, USA) protected by a 3 µm on-line filter unit (Rheodyne, Cotati, CA, USA). A pH 3.8 20 mM phosphate buffer (Solvent A) - acetonitrile (Solvent B) gradient was used. The gradient profile was as follows: 0-3 min, 97% A - 3% B (isocratic); 3-8 min, 85% A - 15% B (linear gradient); 8-12 min, 85% A - 15% B (isocratic). Re-equilibration time of 8 minutes was used. A flow rate of 1.5 ml/min was employed throughout. Eluents were filtered through a 0.45 µm membrane filter (Millipore) and degassed by a constant flow of helium. Detection wavelengths were set as follows: 0-5 min, 220 nm; 5-12 min, 280 nm.

External standard calibration curves

Individual solutions of ephedrine, amphetamine, methamphetamine, MDA, MDMA, MDE, and caffeine in phosphate buffer - acetonitrile (97:3) were prepared at 8 different concentration levels. 20 µl of each standard solution were injected and peak areas obtained were linearly related to

concentration. Concentration ranges and correlation coefficients are reported in Table 1.

## Sample preparation

Each tablet was finely powdered and an aliquot of 50 mg was weighed in a 50 ml volumetric flask. A phosphate buffer - acetonitrile mixture (97:3) was added to volume and the solution was sonicated for 5 minutes. Filtration units Millex LCR were used to remove particles greater than 0.5 μm. 20 μl of the filtered solution were injected in duplicate onto the HPLC column.



## RESULTS AND DISCUSSION

A base-deactivated column, instead of a conventional RP-HPLC column, was chosen in order to: a) improve peak symmetry and therefore reproducibility; b) shorten elution time; and c) reduce the amount of organic solvents in the mobile phase.

An alternative approach was attempted with a conventional C8 silica column and phosphate buffer/acetonitrile mobile phase, but satisfactory results in terms of peak symmetry and separation were achieved only by using a very acidic mobile phase (pH 2.0), thus shortening column stability. Furthermore, a bigger amount of acetonitrile was required to obtain acceptable elution time.

The Suplex pKb100 is a silica based C18 stationary phase in which residual silanols are electrostatically shielded. Vervoort et al. (10) recently reported that this column gives a very good peak symmetry for basic compounds in a pH range of 2.5-6.0 and that the shielding of free silanols is so effective that addition of basic modifiers is not necessary.

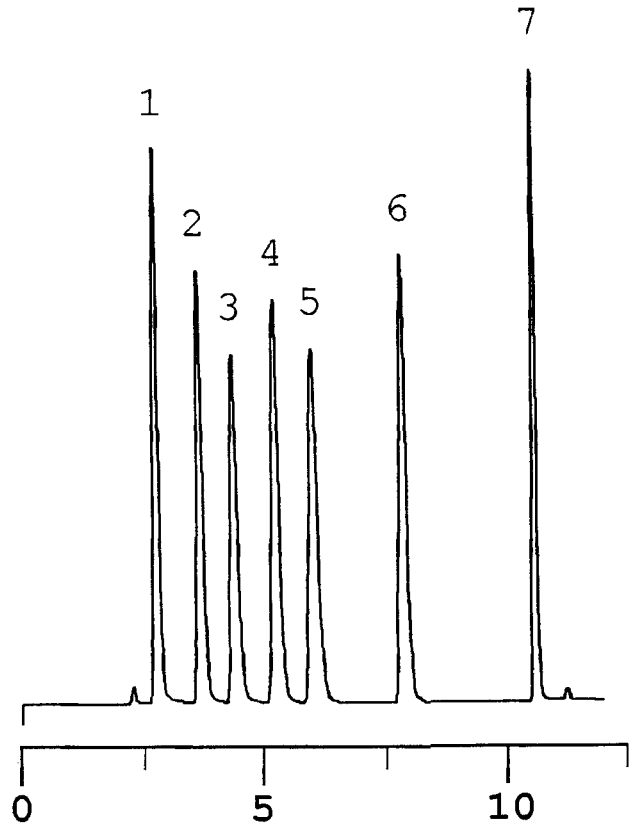


FIGURE 1 : Representative chromatogram showing separation of: (1)ephedrine-102.5 µg/ml; (2)amphetamine-95.7 µg/ml; (3)methamphetamine-100.6 µg/ml; (4)MDA-58.2 µg/ml; (5)MDMA-63.9 µg/ml; (6)MDE-64.2 µg/ml; (7)caffeine-17.5 µg/ml; 20 µl injected; detector sensitivity: 0.2 AUFS.

A representative sample containing the substances of interest was made by mixing accurately weighed amounts of powdered tablets of known composition. This sample was dissolved and injected as previously described. The resulting chromatogram is shown in Figure 1. Retention times ($t_R$), capacity factors (k'), and asymmetry factors ($A_s$) are listed in Table 2.

**TABLE 2**

Chromatographic Characteristics of the Separation

| COMPOUND | $t_R$ (min) | k'(*) | $A_s$ (#) |
|---|---|---|---|
| Ephedrine | 2.73 | 1.02 | 1.84 |
| Amphetamine | 3.62 | 1.68 | 2.15 |
| Methamphetamine | 4.30 | 2.19 | 2.37 |
| MDA | 5.16 | 2.82 | 1.71 |
| MDMA | 5.93 | 3.39 | 1.83 |
| MDE | 7.74 | 4.73 | 1.68 |
| Caffeine | 10.40 | 6.70 | 1.33 |

(*) dead time for the calculation of k' was determined as first baseline disturbance after injection of methanol;

(#) asimmetry factors (As)were calculated at 10% of the peak height using the ratio of the width of the rear and the front sides of the peak.

**TABLE 3**

Accuracy and between-day Precision of the Method

| COMPOUND | ACTUAL (μg/ml) | EXPERIMENTAL(*) (μg/ml) | RECOVERY (%) | RSD(#) (%) |
|---|---|---|---|---|
| Ephedrine | 102.5 | 98.4 | 96.0 | 0.51 |
| Amphetamine | 95.7 | 92.5 | 96.7 | 0.63 |
| Methamphetamine | 100.6 | 97.5 | 96.9 | 0.81 |
| MDA | 58.2 | 57.7 | 99.1 | 0.64 |
| MDMA | 63.9 | 63.5 | 99.4 | 1.05 |
| MDE | 64.2 | 63.7 | 99.2 | 0.55 |
| Caffeine | 17.5 | 17.1 | 97.7 | 0.76 |

(*) average values obtained by determination on 5 separate sample weighings

(#) relative standard deviation obtained from 16 replicate measurements of a representative sample solution

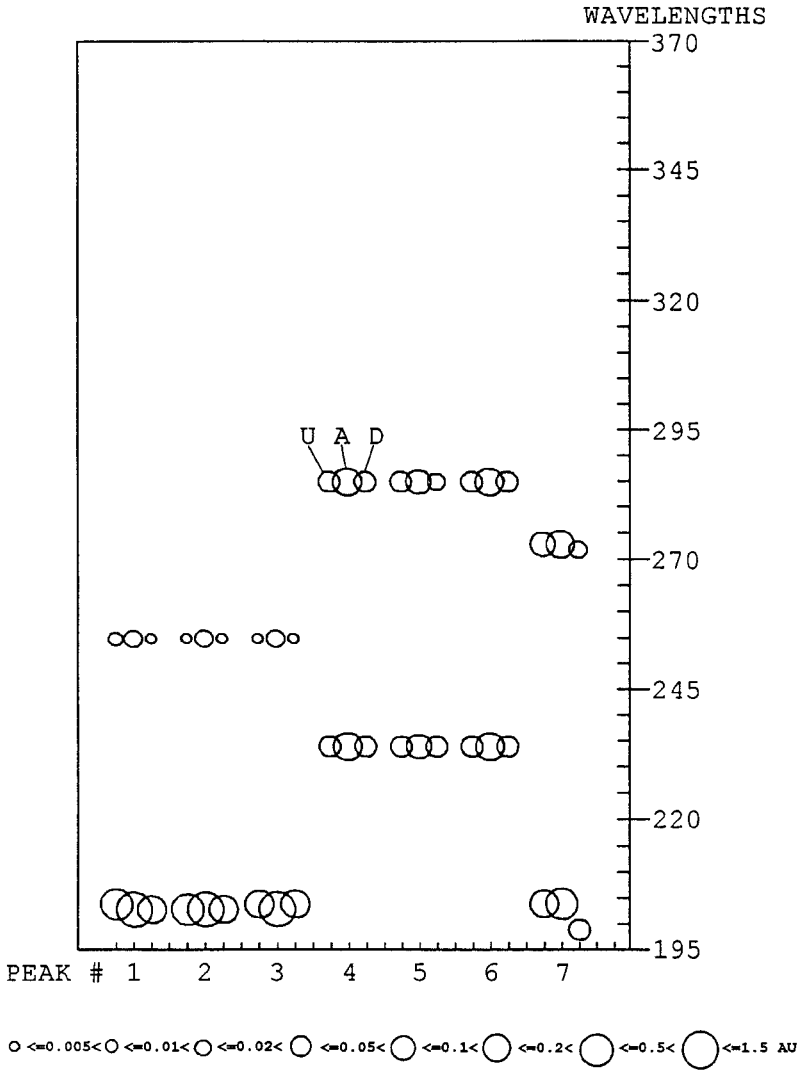


FIGURE 2 : Absorbance profile map of the seven compounds examined: circles represent the intensities of the absorption maxima of the eluting substances. The wavelengths of the absorption maxima at the upslope (U), the apex (A), and the downslope (D) must be coincident for pure peaks.

The between-day precision and accuracy of the proposed method were assessed by the repeated analysis of the representative sample over a period of sixteen days. The results are summarised in Table 3.

A major problem in quantitative analysis is whether a chromatographic peak consists of one or more components, because impurities hidden under the sample compounds falsify results. DAD was used to acquire the UV spectra at the upslope, the apex and at the downslope of each peak. The absorption maxima were plotted peak against wavelength in the spectral absorbance profile map, as it is shown in Figure 2. For pure peaks the wavelengths corresponding to the absorption maxima at the upslope, the apex and at the downslope must be coincident.

A detection wavelength of 280 nm was selected for MDA, MDMA, MDE, and caffeine because of better specificity.



## CONCLUSION

The present HPLC method provides a simple, rapid, precise, and accurate means of assay for amphetamines and 3,4-MDAs in tablets of illicit provenience, even in presence of two common adulterants. Hence it appears to be useful in the routine identification and determination of these substances.

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