

Gas Chromatographic Analysis of Amphetamines from Seized Materials in Organic Solutions Added with Potassium Hydroxide

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Abstract: A simple method to run fast gas chromatographic analyses of some amphetamine derivatives commonly occurring in forensic laboratory activity is proposed. The method is suitable to be applied with a widely used non-polar capillary column in the field of drug analysis, i.e. 5% phenyl – 95% dimethylpolysiloxane reversed phase widebore column. The quality of peaks of amphetamines, usually low with this kind of stationary phase, is improved by simply adding some potassium hydroxide to sample solutions for qualitative purposes, or to the internal standard solution for quantitation. A method to achieve quantitation for amphetamine and “ecstasy” through gas chromatography with flame ionization detector is described and discussed, though not validated.

Key words: Amphetamines; seized materials; forensic analyses; gas chromatography; potassium hydroxide.

INTRODUCTION

The term “amphetamines” and “amphetamine type stimulants” (ATS) refer to a class of compounds virtually derived from amphetamine (1-phenyl-2-aminopropane) through alkylation of the nitrogen atom, as in methamphetamine (1-phenyl-2-methylaminopropane); or substitution of the aromatic ring, as in DMA (2,5-dimethoxyamphetamine), TMA (3,4,5-trimethoxyamphetamine), DOB (4-bromo-2,5-dimethoxyamphetamine), DOE (4-ethyl-2,5-dimethoxyamphetamine), DOM (4-methyl-2,5-dimethoxyamphetamine), MDA (3,4-methylenedioxyamphetamine) and MMDA (5-methoxy-3,4-methylenedioxyamphetamine); or the combination of both, as in MDMA (3,4-methylenedioxy-N-methylamphetamine) and MDEA (3,4-methylenedioxy-N-ethylamphetamine). Amphetamines are central nervous system stimulants, though some of them, depending on substituent groups, show hallucinogenic activities. Several methods are proposed to detect and quantitate amphetamines in seized tablets or in biological matrices, such as immunoassays^[1], thin layer chromatography (TLC)^[2,3], Fourier transform infrared spectrometry (FTIR)^[4], high-performance liquid chromatography (HPLC)^[5,6], gas chromatography with flame ionization detector (GC-FID)^[7,8], gas chromatography hyphenated to mass spectrometry (GC-MS)^[9,10], capillary electrophoresis (CE)^[11].

Capillary gas chromatography (GC) is a widely used technique for routine drug analyses from seized tablets or powders in forensic laboratories. Flame ionization detectors (FID) are easily manageable and unexpensive devices, highly responsive to molecules containing a large number of carbons, such as alkaloids.

Silica-fused widebore capillary columns with a 5% phenyl – 95% dimethylpolysiloxane internal phase are also commonly appreciated in forensic analytical chemistry, achieving separation of a large variety of substances of forensic interest, such as opiates, cocaine (both in the form of free bases and salts, usually chlorhydrates) and canapiates^[12].

Unfortunately, such columns don't seem to suit the structure of the ATS salts, in the form they usually occur in “street drugs” (tablets) and in standards for analysis. Injecting a sample of an ATS salt (typically a chlorhydrate or a sulphate), either coming from a standard or from a seized tablet, often results in broad, unresolved or tailed peaks, unless a proper derivation is applied (a large number of chemical derivation reactions are available^[13]). Extraction from aqueous alkaline solutions of tablets allows to get the free bases for qualitative purposes, but not for quantitation, since unpredictable amounts of substances may be lost along this procedure. Moreover, chemist officers involved in drug abuse prosecution are interested in achieving one-step, yet acceptably complete, dissolution of the active

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agent in the tablets, in order to quickly fulfill the requests of the inquiring authority.

Thus, we have developed a method which should allow the analyst to perform a good separation of amphetamine and three related molecules, commonly occurring in the seized tablets, by using the same columns for common alkaloids. We selected the compounds whose structures are showed in Figure I: (a) amphetamine, (b) 3,4-methylenedioxy-N-methylamphetamine (MDMA, often referred to as "ecstasy" or "Adam"), (c) N-methylamphetamine ("meth" or "shaboo") and (d) 3,4-methylenedioxy-N-ethylamphetamine (MDEA, also called "Eve"). These four molecules commonly occur in the form of hydrochloride salts, sometimes sulphates (anions are identified by traditional wet-chemistry tests). Following the procedure described below, the analyst will be allowed to keep the same column while running a sequence of analyses of different drugs and to avoid the derivatization of samples. This procedure has been applied in order to plan two methods suitable for quantifying amphetamine and MDMA in "street drug" tablets.

MATERIALS AND METHODS

First, we found that adding a few drops of a 1 N solution of potassium hydroxide in methanol to sample solutions prepared each by dissolving 30-50 mg of a grinded "ecstasy" tablet and 10 mg of lidocaine in 10 ml of methanol, greatly improved the sharpness of the gas chromatographic peaks (Figure II). This occurrence was observed both with 5% phenyl – 95% dimethylpolysiloxane and 17% phenyl – 83% dimethylpolysiloxane internal phase capillary columns. In some cases, the peak sharpness increased dramatically: Figures III and IV show what happens to the totally unresolved peak of amphetamine hydrochloride when potassium hydroxide is added. Another commonly misused molecule is N-methylamphetamine, often in the form of white crystals: the results of adding potassium hydroxide to its methanolic solution is shown in Figures V and VI. Starting from these observations, an alkaline solution of lidocaine as internal standard in methanol was prepared, both to get the standard calibration solutions and to dissolve the weighted tablets for quantifying the active agent. Of course, lidocaine is suitable for being used as internal standard provided it is not part of the tablets under study.

Preparation of the Internal Standard Solution: 500 mg of Sigma lidocaine hydrochloride are stirred with 200 ml of methanol in an Erlenmeyer flask until dissolution, then added with 12.5 ml of a 1 N solution of potassium hydroxide in methanol (obtained by

dissolving 56.1 g of Carlo Erba Analyticals potassium hydroxide pellets in methanol to the volume of 1 l). This solution is carefully transferred to a 500 ml volumetric flask and methanol is added to the volume notch, thus obtaining a 0.025 N potassium hydroxide solution in methanol, containing 1.0 mg/ml of lidocaine hydrochloride.

In the present work, this solution will be referred to as "LK solution".

Preparation of the Calibrating Solutions for 3,4-methylenedioxy-n-methylamphetamine: 20 mg of Sigma S(+)-3,4-methylenedioxy-N-methylamphetamine hydrochloride (MHCl) are dissolved with 5 ml of LK solution in a 10 ml volumetric flask, and stirred by vortex; then, more LK solution is added until the volume notch, thus obtaining a 2.0 mg/ml MHCl, 1.0 mg/ml lidocaine hydrochloride and 0.025 N potassium hydroxide solution in methanol. This solution represents the "first level" calibration standard for the method (see below), as well as the starting solution from which the "second level" and the "third level" calibration standards are obtained by progressive dilutions with "LK solution".

Preparation of the Calibrating Solutions for Amphetamine: 50 mg of Sigma d,l-amphetamine sulphate ($A_2H_2SO_4$) are dissolved with 15 ml of LK solution in a 25 ml volumetric flask, and stirred by vortex; then, more LK solution is added until the volume notch, thus obtaining a 2.0 mg/ml $A_2H_2SO_4$, 1.0 mg/ml lidocaine hydrochloride and 0.025 N potassium hydroxide solution in methanol. This solution represents the "first level" calibration standard for the method, as well as the starting solution from which the "second level" and the "third level" calibration standards are obtained by progressive dilutions with "LK solution".

Calibration of Instruments: A Perkin Elmer 8500 Gas Chromatograph with Flame Ionization Detector, managed by PE Turbochrom Navigator 4.1 software, equipped with a SGE BP5 30m x 0.32mm ID x 0.25mm column (equipment # 1), and an Agilent 6890 System Gas Chromatograph with Flame Ionization Detector, managed by Agilent Chemstation software, equipped with a J&W HP-5 30m x 0.32mm ID x 0.25mm column (equipment # 2), were both calibrated for MDMA and amphetamine quantitation, using different conditions for the two molecules.

Moreover, a Perkin Elmer 8600 Gas Chromatograph with Flame Ionization Detector equipped with a J&W DB-17 10m x 0.32mm ID x 0.25mm column (equipment # 3) was tested only for qualitative purposes, though not calibrated.

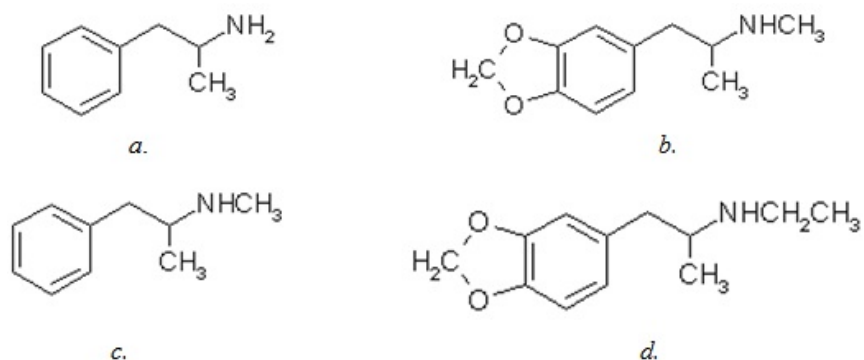


Fig. I: Chemical structures of amphetamine (a), 3,4-methylenedioxy-N-methylamphetamine (b), N-methylamphetamine (c) and 3,4-methylenedioxy-N-ethylamphetamine (d)

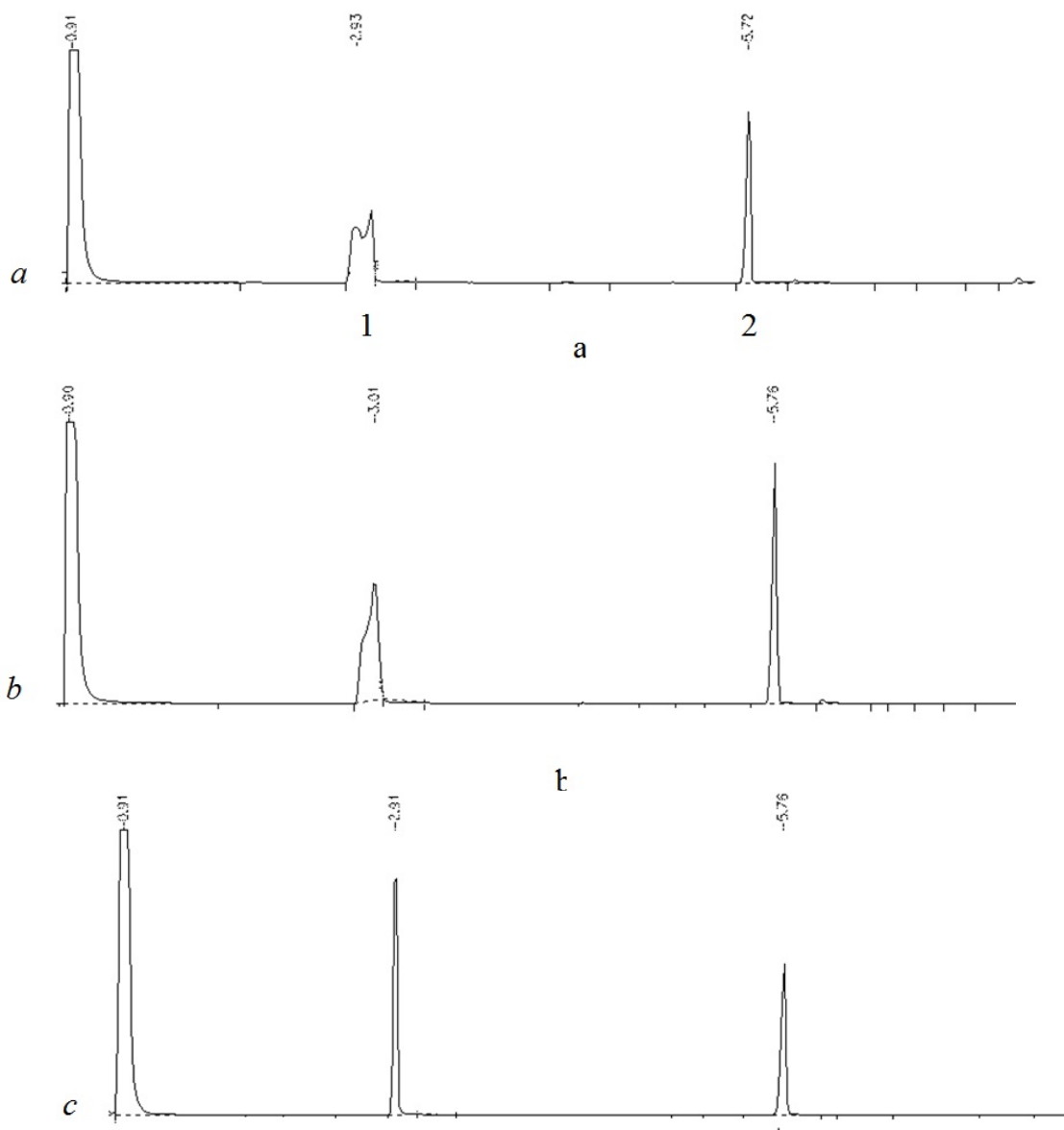


Fig. II: Chromatograms of an MDMA (peak #1) tablet in lidocaine (peak #2) solution, before (a) and after adding a little amount of potassium hydroxide (increasing from b to c).

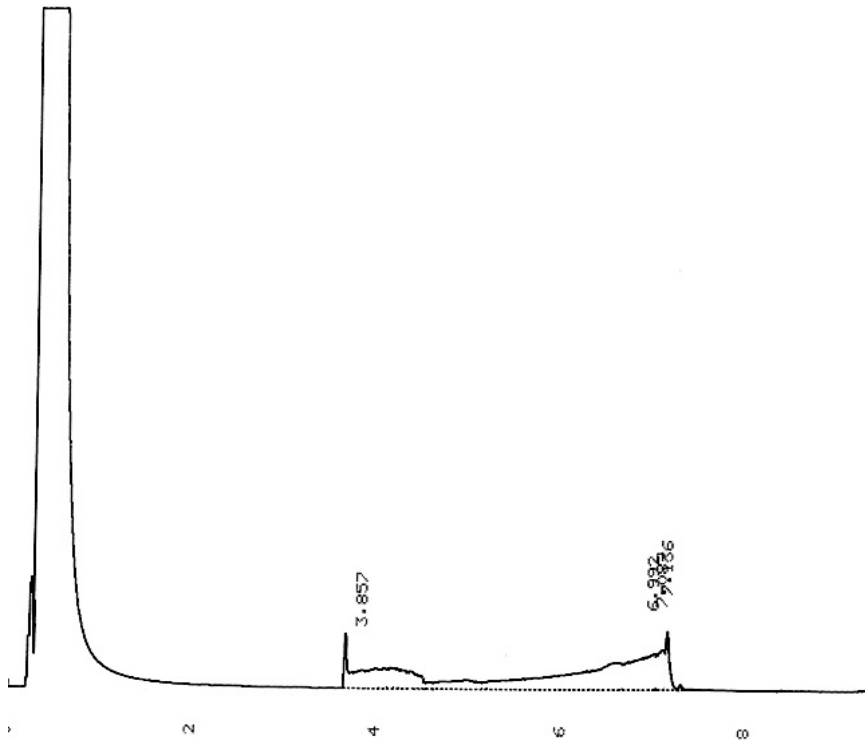


Fig. III: Chromatogram of amphetamine hydrochloride in methanol (17% phenyl – 83% dimethylpolysiloxane internal phase capillary column).

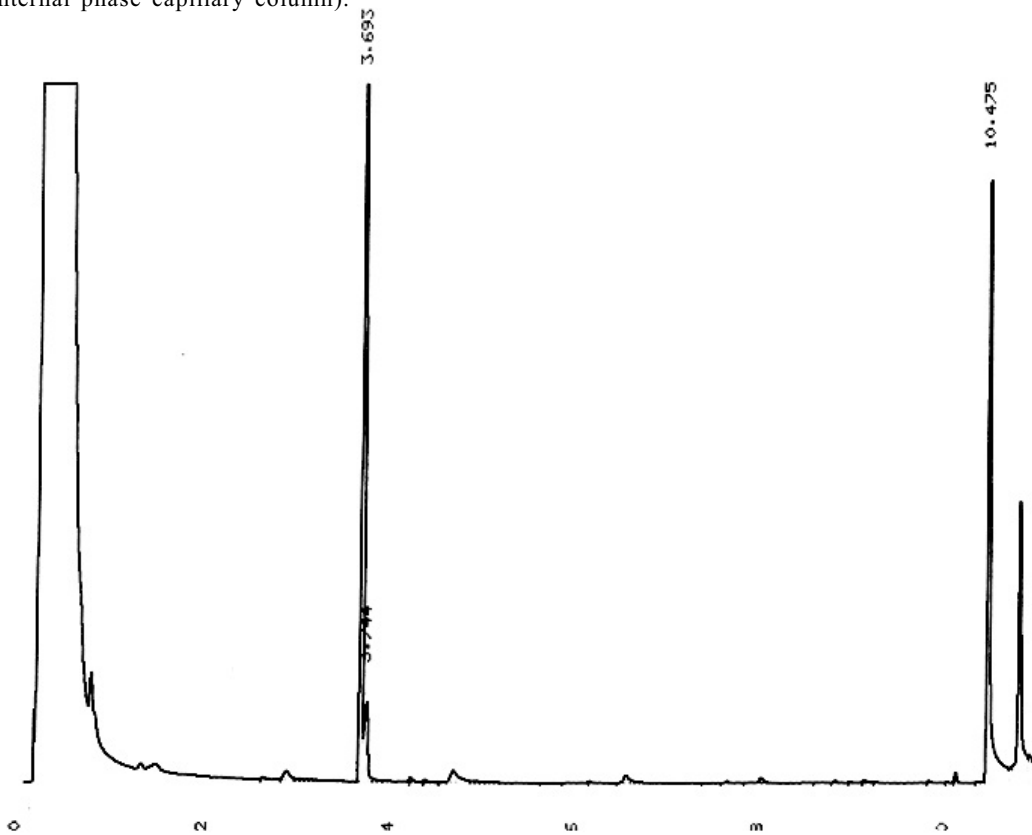


Fig. IV: Chromatogram of amphetamine hydrochloride in LK solution (same conditions than Fig. III). Peak at 10.475 is lidocaine.

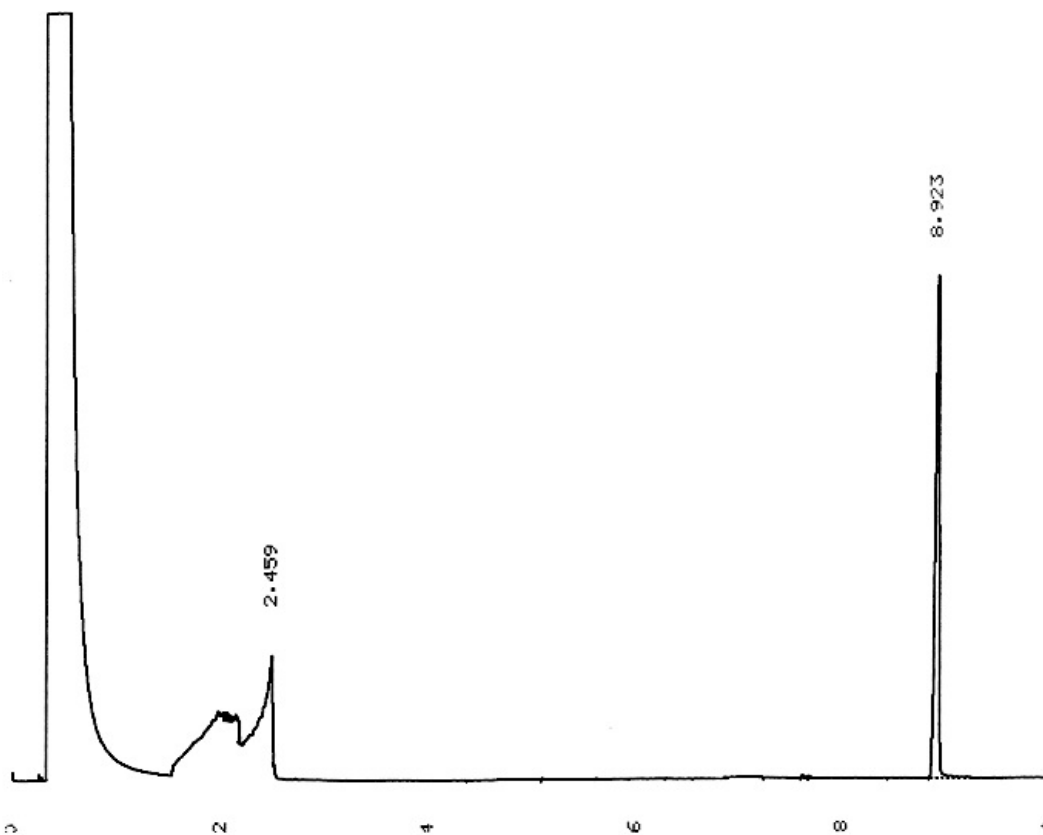


Fig. V: Chromatogram of N-methylamphetamine hydrochloride in methanol (17% phenyl - 83% dimethylpolysiloxane internal phase capillary column).

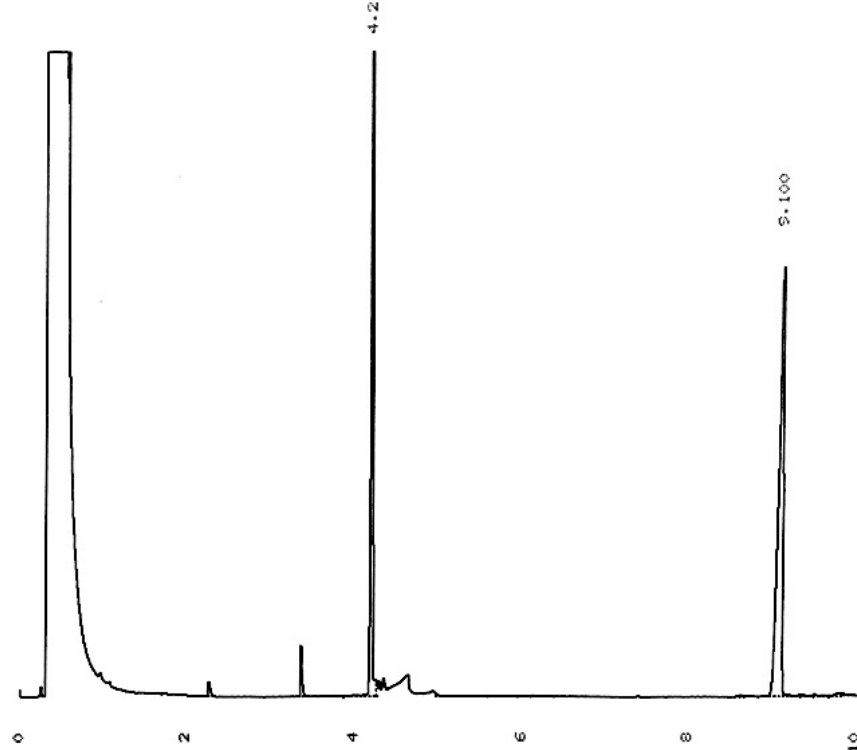


Fig. VI: Chromatogram of N-methylamphetamine hydrochloride in LK solution (same conditions than Fig. V).

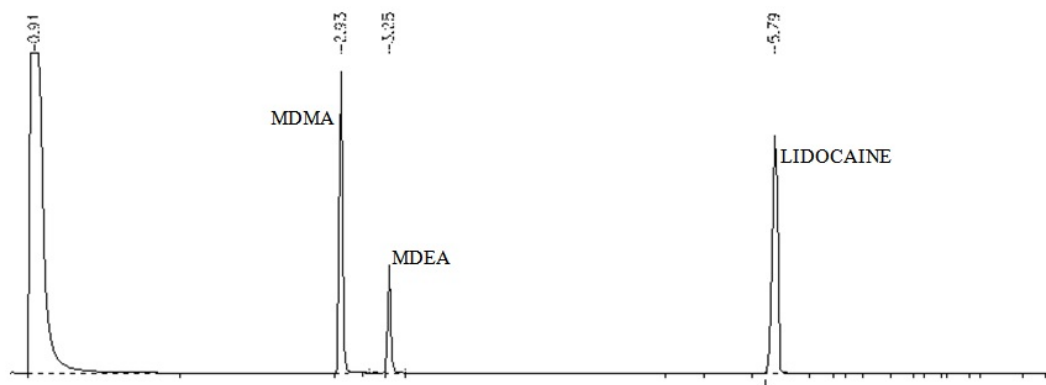


Fig. VII: Separation between MDMA and MDEA, obtained with equipment # 2.

1. GC/FID Perkin Elmer 8500: MDMA method. Initial temperature 150 °C, isotherm for 1 min, ramp rate 13 °C/min up to 240 °C, ramp rate 15 °C/min up to 300 °C, isotherm for 1 min (total run time 12.9 min). Carrier gas He. Injector temperature 300 °C. Detector temperature 320 °C. Constant pressure 20.0 psi. Split ratio 50:1.

Amphetamine method. Initial temperature 90 °C, ramp rate 10 °C/min up to 130 °C, ramp rate 15 °C/min up to 300 °C, isotherm for 1 min (total run time 16.3 min). Carrier gas He. Injector temperature 300 °C. Detector temperature 320 °C. Constant pressure 20.0 psi. Split ratio 50:1.

2. GC/FID Agilent 6890 System: MDMA method. Initial temperature 150 °C, isotherm for 1 min, ramp rate 13 °C/min up to 240 °C, ramp rate 15 °C/min up to 300 °C, isotherm for 1 min (total run time 12.9 min). Carrier gas He. Injector temperature 300 °C. Detector temperature 320 °C. Constant pressure 20.0 psi. Split ratio 50:1.

Amphetamine method. Initial temperature 90 °C, ramp rate 10 °C/min up to 130 °C, ramp rate 15 °C/min up to 300 °C, isotherm for 1 min (total run time 16.3 min). Carrier gas He. Injector temperature 300 °C. Detector temperature 320 °C. Constant pressure 20.0 psi. Split ratio 50:1.

3. GC/FID Perkin Elmer 8600: MDMA method. Initial temperature 150 °C, isotherm for 1 min, ramp rate 7 °C/min up to 220 °C, ramp rate 15 °C/min up to 300 °C, isotherm for 1 min (total run time 17.3 min). Carrier gas He. Injector temperature 300 °C. Detector temperature 320 °C. Constant pressure 16.0 psi. Split ratio 50:1.

Amphetamine method. Initial temperature 70 °C, ramp rate 10 °C/min up to 130 °C, ramp rate 15 °C/min up to 300 °C, isotherm for 1 min (total run time 18.3 min). Carrier gas He. Injector temperature

300 °C. Detector temperature 320 °C. Constant pressure 16.0 psi. Split ratio 50:1.

RESULTS AND DISCUSSION

For both equipments # 1 and 2, the MDMA method separates MDMA and MDEA, that are sometimes found as mixtures in “street drug” tablets. On the other hand, the amphetamine method is recommended for the isolation of amphetamine and N-methylamphetamine.

With the equipment # 3, too, the proposed methods offer good separation between MDMA and MDEA on one side, and between amphetamine and N-methylamphetamine on the other.

As shown in Figure VIII for the equipment # 2 and its related software, a calibration curve of good linearity in the selected range from 0.5 to 2.0 mg/ml is achieved for MDMA. Though we chose not to force the curves through the origin of axes, they seem to pass through it anyway, showing a low baseline noise. This range usually fits the amount of MDMA in an average weight tablet containing an average percentage of active molecule. In other words, 25 to 100 mg of a 20 % w/w MDMA tablet (a very common percentage in “street drug” tablets) may be dissolved in a 10 ml volumetric flask to fit the range of calibration, that is from 10 % to 40 % of a typical 250 mg tablet (a very common weight of “street drug” tablets). On the other hand, a 50 mg amount of a tablet dissolved in a 10 ml volumetric flask suits percentages of active molecule from 10 to 40.

The potassium hydroxide normality of the “LK solution” was chosen to be about twice the total normality of the hydrochloride species present in the upper level calibration standard. For example, in 10 ml of a 2.0 mg/ml MDMA hydrochloride (m.w. 229.7) and 1.0 mg/ml lidocaine hydrochloride (m.w. 270.8) solution, the total number of hydrochloride milliequivalents are:

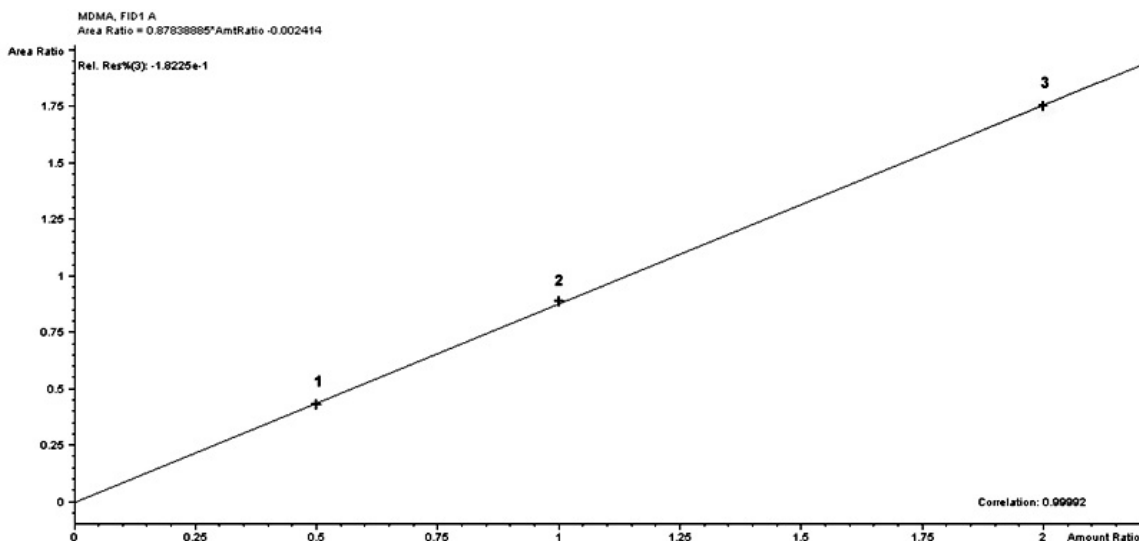


Fig. VIII: Calibration curve for 3,4-methylenedioxy-N-methylamphetamine in equipment # 2.

$$20/229.7 + 10/270.8 = 0.087 + 0.037 = 0.124$$

As regards amphetamine sulphate (m.w. 368.5):

$$20*2/368.5 + 10/270.8 = 0.108 + 0.037 = 0.145$$

The same volume of 0.025 N potassium hydroxide (m.w. 56.1) contains the following number of milliequivalents of alkali:

$$10*0.025 = 0.25.$$

Of course, the hydrochloric acid corresponding to any lower concentration in MDMA (or amphetamine) hydrochloride is certainly neutralized by the added hydroxide. The exceeding potassium hydroxide is able to neutralize other hydrochloride species that may be present in the tablets, thus fitting the purpose of this work of developing a practical method, suitable to be applied in forensic laboratories to a large part of “street drug” tablets, in a large range of sample concentrations.

For practical analyses, we suggest to weigh about 50 mg of the finely grinded tablet in a 10 ml volumetric flask, then fill it to the volume notch with the LK solution and shake the mixture by a vortex. We followed this procedure for some tablets, as reported in Figure VII. The amount of active molecule in a “street drug” tablet containing from 10 % to 40 % w/w of MDMA hydrochloride should fall within the calibration range; lower concentrations are infrequent, while higher concentrations will require a further dilution with LK solution (as a matter of fact, we did not investigate the linearity range of the method, which may be even

wider). On another part of the grinded tablet we usually apply traditional wet-chemistry assays to investigate the presence of the chloride or sulphate anions.

Conclusions: Adding a small amount of a solution of potassium hydroxide in methanol to the solvent results in the improvement of gaschromatographic peaks’ sharpness of some amphetamine related molecules in street drug tablets, when a non-polar capillary column is employed. We found that a 0.025 N potassium hydroxide solution in methanol, containing 1.0 mg/ml of lidocaine hydrochloride, is suitable for being employed as internal standard solution for quantitation of amphetamine and MDMA in most tablets coming from the illicit trade. This procedure allows the chemists to run fast gas chromatographic analyses of amphetamines using the same columns employed in most examinations of the commonly seized alcaloids (opiates, canapiates, cocaine).

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