TECHNICAL NOTE

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A Convenient Derivatization Method for the Determination of Amphetamine and Related Drugs in Urine

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ABSTRACT: The most commonly abused CNS stimulant in Sweden is amphetamine followed by phenmetrazine. Methamphetamine and phentermine are rarely seen but still of interest. This paper describes a rapid and sensitive method for the analysis of amphetamine, methamphetamine, phentermine, and phenmetrazine in urine using gas chromatography with nitrogen sensitive detection (GC-NPD). The method also qualitatively determines ephedrine and norephedrine. The derivatization was carried out at room temperature with methyl chloroformate to form the corresponding carbamates. Other chloroformate analogues were also tested. Because methyl chloroformate is relatively stable in the presence of water the extraction and derivatization were combined in one step. A concentration step was not necessary to achieve sufficient sensitivity. The recovery was more than 83% for all analytes. The LOQ was 0.05, 0.03, 0.07 and 0.01 (μg/mL urine) for amphetamine, methamphetamine, phentermine and phenmetrazine respectively. The cut-off was set at 0.2 μg/mL. The within-day and between-day relative standard deviation (RSD) for amphetamine were 2.2% ($n = 9$) and 4.7% ($n = 5$) respectively. There was a good quantitative correlation ($r^2 = 0.995$) between GC-NPD using chloroformate derivatives and gas chromatography-mass spectrometry (GC-MS) using trifluoroacetic anhydride (TFA) as derivatizing agent for the determination of amphetamine in authentic samples.

KEYWORDS: forensic science, drug chemistry, drug analysis, controlled substances, derivatization, amphetamine, methamphetamine, phentermine, phenmetrazine

The applications for derivatizing agents that can be used under aqueous conditions have increased during recent years. Chloroformates have been used for this purpose, especially for low mass biogenic amines (1,2), aminoacids and catecholamines (3–6). Aliphatic hydroxy groups do not react with these types of derivatization reagents. The relatively unstable phenolic chloroformate derivatives can be alkylated or converted to silylethers to gain stability and sensitivity (4,6). Menthyl chloroformate has been used for detection and separation of the stereoisomeres of amphetamine and methamphetamine in urine but the derivatives were produced under anhydrous conditions after the extraction (7). The aim of this study was to develop a rapid and sensitive procedure for the determination of amphetamine, methamphetamine, phentermine and phenmetrazine in urine using gas chromatography with nitrogen sensitive detection. The structure of the derivatives was confirmed by gas chromatography-mass spectrometry.

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Materials and Methods

Reagents

Amphetamine-sulphate was purchased from Apoteksbolaget AB, Sweden. Methamphetamine hydrochloride, phentermine hydrochloride, ethylamphetamine hydrochloride and phenmetrazine hydrochloride were obtained from the National Laboratory of Forensic Sciences, Sweden. Ephedrine hydrochloride and norephedrine sulphate were purchased from Sigma and phenylethylamine hydrochloride was purchased from Aldrich. Amphetamine-d_5 was purchased from Radian. Methyl chloroformate, ethyl chloroformate and isobutyl chloroformate were purchased from Fluka. Trifluoroacetic anhydride was purchased from Aldrich. Sodium carbonate, sodium hydrogencarbonate, ammonium carbamate, sodium hydroxide, potassium hydroxide, hydrochloric acid, methanol and isooctane were of p.a. grade and purchased from Merck. Acetonitrile (far UV grade) was obtained from Fisons.

Standards

Standard solutions were prepared by weighing the appropriate amount of drug and diluting with methanol. The stock solutions were stored frozen.

Methyl-N-(1-phenyl-2-propyl)-carbamate *(Amphetamine methylcarbamate)* was synthesized in isooctane using methyl chloroformate and amphetamine in equimolar amounts. After the reaction was completed the organic phase was washed with hydrochloric acid and water. The organic phase was evaporated and the purity of the oil was determined by GC, high performance liquid chromatography (HPLC) and GC MS. An aliquot of the oil was weighed and diluted with isooctane. This stock solution was used for recovery studies of amphetamine.

Instrumentation

The GC analysis was performed on a Hewlett Packard 5890 GC equipped with a NPD, a 7673A autosampler and a HP 3396

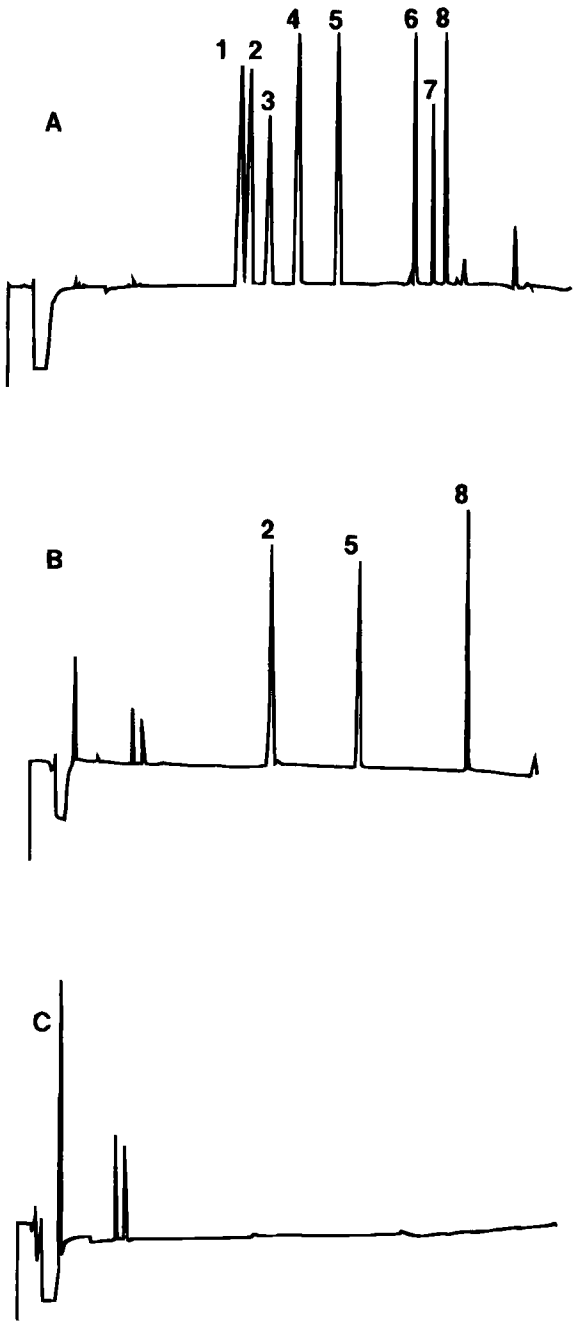

FIG. 1—*Gas chromatography of amines as their methyl chloroformate derivatives* A) *Standard sample, 1, phenylethylamine, 2, amphetamine, 3, phentermine 4, methamphetamine, 5, ethylamphetamine (IS), 6, norephedrine, 7, ephedrine, 8, phenmetrazine.* B) *Sample from drug user.* C) *Blank sample.*

integrator. The column was a J&W 15 m DB-5 with i.d. 0.25 mm and 1.0 μm film thickness. The carrier gas was helium with a column head pressure of 11.5 psi. Splitless injection was used with a splitless time of 1 minute. Injector and detector temperature were 210°C and 300°C respectively. The oven temperature was held at 85°C for 1 minute and then increased at 20°C/min to 165°C, held there for 6 minutes, then 25°C/min to 290°C where the temperature was held for 1 minute.

The GC-MS analysis was performed on a Hewlett Packard 5980 GC interfaced to a 5972A MSD. The column was a 30 m HP-5 MS, i.d. 0.25 mm and a film thickness of 0.25 μm. Helium was used as carrier gas and the flow was set at 1.0 mL/min through the whole temperature program. Splitless injection was used with a splitless time of 1 minute. Injector and MS interface temperature were 210°C and 280°C respectively. The oven temperature was held at 85°C for one minute and then increased at 28°C/min to 180°C and then increased at 35°C/min to 280°C.

The HPLC analysis was performed with a Waters M590 pump and a 150 mm (4.6 mm i.d.) Hamilton 5 μm PRP-1 column. Samples were manually injected using a Rheodyne 7125 injector and amphetamines and their derivatives were detected with a Waters 480 UV-detector at 230 nm. The mobile phase was acetonitrile:ammonium carbamate (1:1) at a flow rate of 1.0 mL/minute.

Extraction and Derivatization

Samples for standard curves were prepared from drug free urine by addition of standard solutions. Authentic samples were first analyzed by the Abbott TDx as proposed by the manufacturer. If the concentration from the TDx analysis was greater than 6 μg/mL, the urine was diluted ten fold. 0.2 mL of each sample was used for analysis for both the GC and GC-MS methods.

GC Method (Chloroformates)—The sample was transferred to a 10 mL screwcapped glasstube and the pH was adjusted to 9 by addition of 1 M sodium carbonate buffer. Internal standard (1.0 μg of ethylamphetamine) and 0.5 mL isooctane were added. 50 μL of methyl chloroformate was then added and the mixture was shaken at room temperature for 10 minutes.

After the derivatization and extraction the organic phase was transferred to a new tube. 0.2 mL of methanol saturated with potassium hydroxide was added to destroy any unreacted methyl chloroformate as described by Hartvig et al. (8).

0.5 mL of 2 M potassium hydroxide was added and an aliquot of the isooctane was transferred to micro vials. 1 μL was injected into the GC.

GC-MS Method (TFA)—The sample was transferred to a 10 mL screwcapped glasstube and 200 μL 2 M sodium hydroxide was added. Internal standard, (0.2 μg amphetamine-d5) and 2 mL isooctane were added and the mixture was shaken for 5 minutes. After centrifugation the organic phase was transferred to a new tube and evaporated to dryness under a stream of nitrogen at room temperature. Trifluoroacetic anhydride, 100 μL, was added and the mixture was heated at 60°C for 15 minutes (9,10). The reaction mixture was evaporated to dryness and the residue dissolved in 100 μL isooctane. 1 μL was injected into the GC-MS.

Recovery Studies

Derivatization and extraction efficiencies were determined using HPLC with UV-detection. Standard samples were derivatized in carbonate buffer and after different times a 50 μL aliquot was injected. The peaks for the derivatized analytes were compared to those for unchanged drug to determine the derivatization yield. To estimate the extraction recovery 50 μL of the aqueous phase from samples that were derivatized and extracted by the chloroformate method was injected and peak areas were compared to those from the nonextracted standards.

Total recovery for amphetamine was also studied with GC using synthesized amphetamine methyl carbamate compared to standard samples of amphetamine which were extracted and derivatized. Limit of detection (LOD) and limit of quantitation (LOQ) were

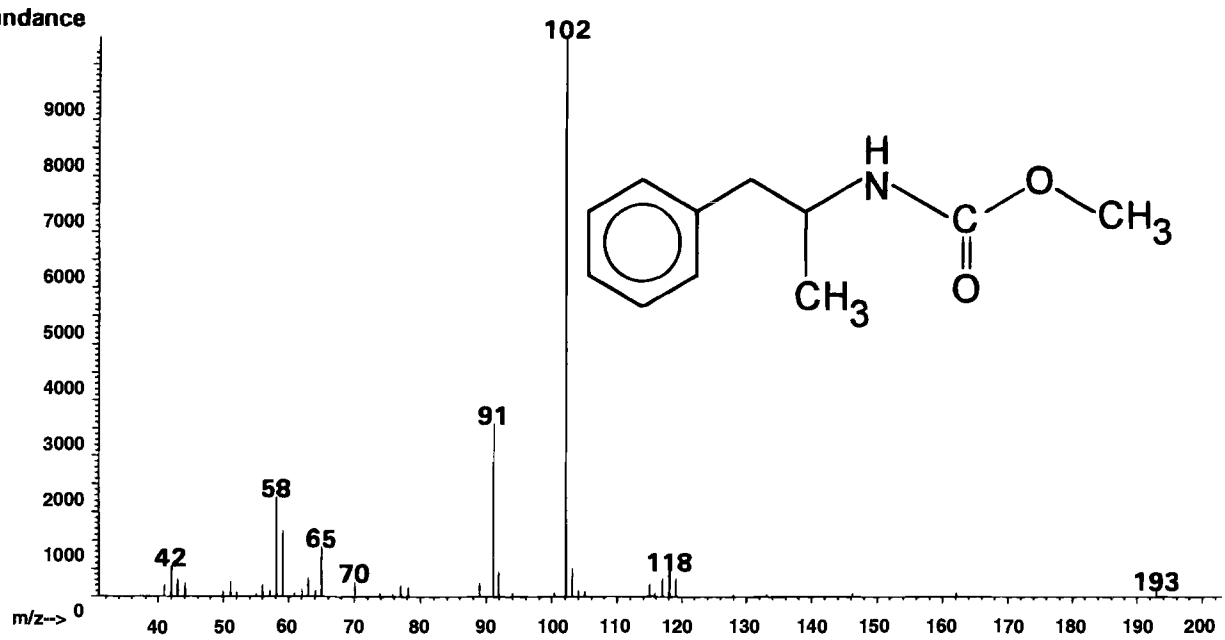

FIG. 2—*Mass spectra of amphetamine derivatized with methyl chloroformate. M+ = m/z 193.*

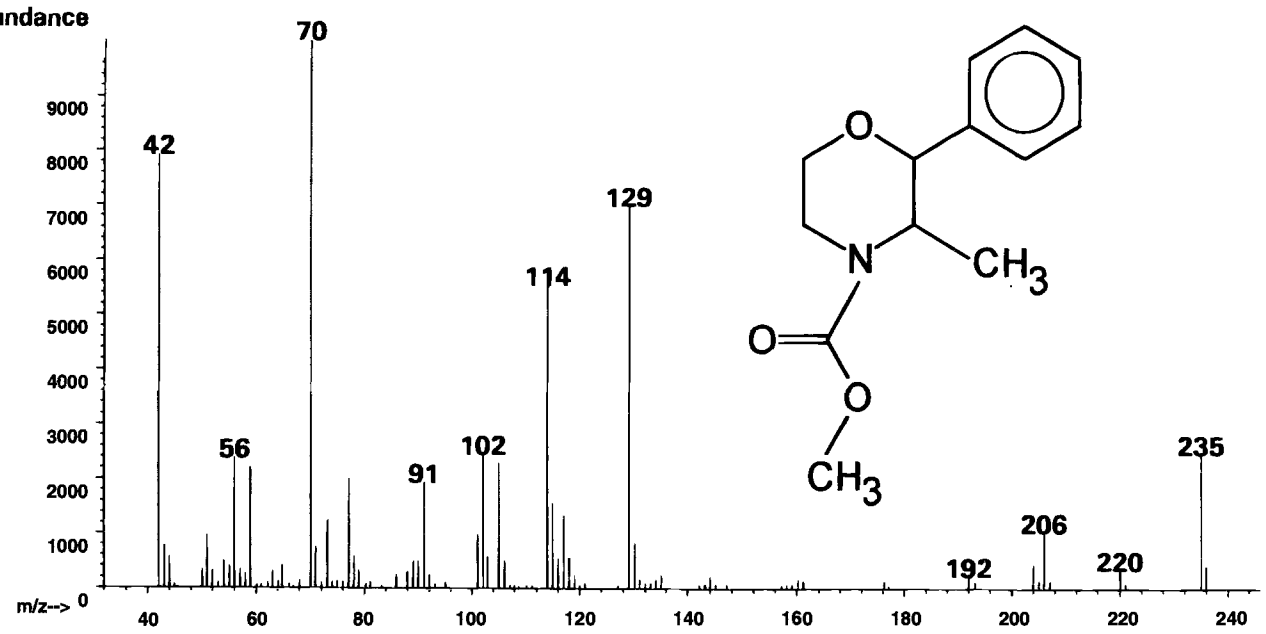

FIG. 3—*Mass spectra of phenmetrazine derivatized with methyl chloroformate. M+ = m/z 235.*

determined accordingly to Knoll (11). This method uses the noise fluctuations in a preselected chart interval of blank specimens to estimate the LOD and LOQ.

Results and Discussion

One of the problems to overcome was to achieve optimal resolution between the endogenously formed phenylethylamine and amphetamine. Phenylethylamine is generated in the body by decarboxylation of phenylalanine. During development of the method methyl-, ethyl- and isobutyl chloroformate were compared as derivatizing agents. The best resolution was achieved with derivatives formed with methyl chloroformate. Figure 1 shows chromatograms from standards, a sample containing amphetamine and phenmetrazine, and a blank specimen. GC-MS was performed on the different derivatives to obtain mass spectra. Spectra of amphetamine and phenmetrazine are shown in Figs. 2 and 3.

The derivatization reaction is quantitative within 10 minutes at room temperature for all analytes except phentermine. For phentermine, a reaction time of approximately 30 minutes was necessary to achieve 100% yield of the derivative. An explanation might be that the second methylgroup in α-position causes steric problems. The derivatization and extraction recoveries of the analytes are shown in Table 1. Recovery for the internal standard was close to

TABLE 1—*LOD, LOQ, derivatization and extraction recovery of the analytes.*

Analyte	LOD (μg/mL)	LOQ (μg/mL)	Derivatization recovery (%)	Extraction recovery (%)
Amphetamine	0.02	0.05	100	96
Methamphetamine	0.01	0.03	100	97
Phentermine	0.02	0.07	84	99
Phenmetrazine	0.004	0.01	100	98

100%. The total recovery of amphetamine was estimated using the synthesized amphetamine carbamate and found to be approximately 98%.

An evaporation step or washing procedure was necessary to remove excess reagent and sideproducts that interfered with the analysis or caused rapid degradation of the NP-detector. Also peaks corresponding to derivatized amphetamine could occur if underivatized amphetamine was injected after an unwashed sample. As evaporation of the solvent extract was not necessary in order to obtain sufficient sensitivity and since it was more time consuming than the washing step, it was omitted.

Limit of detection and limit of quantitation for the analytes are presented in Table 1. The within and between day relative standard deviation for amphetamine were 2.2% (n = 9) and 4.7% (n = 5), respectively.

A total of 44 samples that previously had been analyzed with our present GC-MS method for amphetamines were also analyzed with the GC method and the quantitative results were compared.

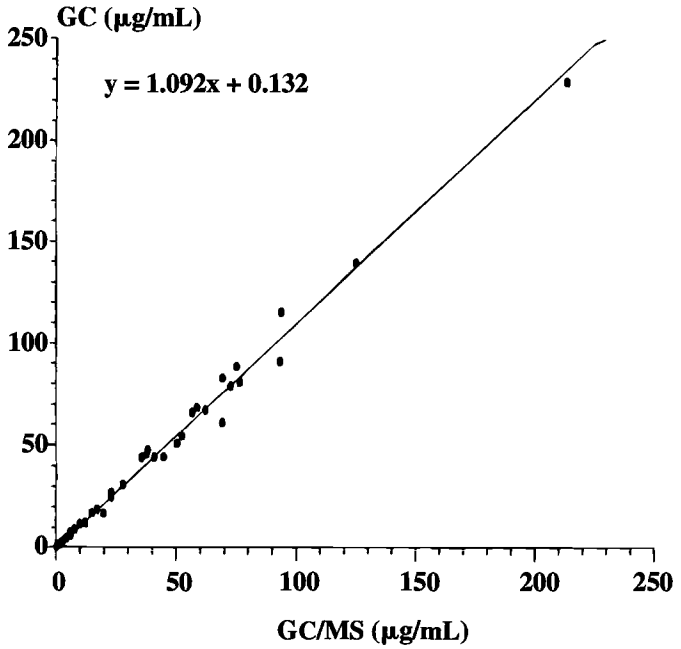

FIG. 4—*Comparison of GC method using methyl chloroformate and GC-MS method using trifluoroacetic anhydride as derivatizing agent. Number of samples: 44.* y = *1.092x + 0.132 Correlation coefficient: 0.995.*

The concentration range was 0.3–230 μg/mL. A correlation scatter plot is shown in Fig. 4. The wide concentration range in authentic samples made it necessary to dilute samples even though we used only 0.2 mL of urine for analysis.

Conclusion

This investigation demonstrates that methyl chloroformate can be used as a derivatizing reagent for the determination of amphetamines, including phenmetrazine, in urine. The advantages compared to the more common procedures using fluorinated anhydrides or silylating reagents are that no heating is required and that the derivatization can take place under aqueous conditions during the extraction, thus no evaporation steps are necessary. The procedure is rapid and sensitive with a high reproducibility. A good correlation was found when compared with GC-MS analysis using trifluoroacetic anhydride as derivatizing agent. The method is a good alternative when GC-MS is not available or not required.

References

(1) Ahnfelt NO, Hartvig P. Gas chromatographic analysis of primary and secondary amines after derivatization with chloroformates in buffered aqueous solution. Acta Pharmaceutica Suecica 1980;17:307–18.

(2) Lundh T, Åkesson B. Gas chromatographic determination of primary and secondary low-molecular-mass aliphatic amines in urine using derivatization with isobutyl chloroformate. J Chromatography 1993;617:191–6,

(3) Wang J, Huang Z-H, Gage DA, Watson JT. Analysis of amino acids by gas chromatography-flame ionization detection and gas chromatography-mass spectrometry: simultaneous derivatization of functional groups by an aqueous-phase chloroformate-mediated reaction. J Chromatography A 1994;663:71–8.

(4) Gyllenhaal O, Johansson L, Vessman J. Gas chromatography of epinephrine and norepinephrine after derivatization with chloroformates in aqueous media. J Chromatography 1980;190:347–57.

(5) Husek P, Huang Z-H, Sweely CC. Gas chromatographic determination of amines, aminoalcohols and acids after treatment with alkyl chloroformates. Analytica Chimica Acta 1992;259:185–92.

(6) De Jong APJM, Cramers CA. Derivatization of catecholamines in aqueous solution for quantitative analysis in biological fluids. J Chromatography 1983;276:267–78.

(7) Hughes RO, Bronner WE, Smith ML. Detection of amphetamine and methamphetamine in urine by gas chromatography/mass spectrometry following derivatization with (=)-menthyl chloroformate. J Anal Toxicol 1991;15:256–9.

(8) Hartvig P, Handl W, Vessman J, Svahn CM. Electron capture gas chromatography of tertiary amines as pentafluorobenzyl carbamates. Anal Chem 1976;48:390–3.

(9) Zeman A, Wirotama IPG. Identifizierung von Aminen. II. Capillargas-chromatographie-massenspektrometri (GC-MS) von trifluoroacetylderivaten (TFA-amine). Zeitschrift für Analytische Chemie 1969;247:158–63.

(10) Ånggård E, Hankey A. Derivatives of sympathomimetic amines for gas chromatography with electron capture detection and mass spectrometry. Acta Chemica Scandinavica 1969;23:3110–19.

(11) Knoll JE. Estimation of the limit of detection in chromatography. J Chromatographic Sci 1985;23:422–5.

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