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Procedure for Determination of Amphetamine and Methamphetamine in Urine by GC/MS Method

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Procedure for Determination of Amphetamine and Methamphetamine in Urine by GC/MS Method

Summary : *In the present study a procedure for the determination of amphetamine and methamphetamine in urine by Gas Chromatography - Mass Spectrometry (GC/MS) was presented. Amphetamine and methamphetamine were extracted by solid-phase extraction (SPE) and trifluoroacetic anhydride (TFA) was used as the derivatization reagent. The derivatized extracts were analyzed by GC/MS which operated in the selected ion monitoring (SIM) mode. Deuterated analogs of the analytes were used as internal standards. Recovery rates of 93.6% and 94.1% for amphetamine and methamphetamine respetcively were obtained.*

Key words: *Amphetamine, Methamphetamine, GC/MS method*

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İdrarda GC/MS metodu ile amfetamin ve metamfetamin tayini

Özet : *Bu çalışmada GC/MS metodu ile idrarda amfetamin ve metamfetamin için bir tayin yöntemi sunulmuştur. Amfetamin ve metamfetamin katı faz ekstraksiyonu (SPE) ile ekstrakte edildikten sonra trifluoroasetik anhidrid (TFA) ile derivatize edilmiş ve derivatize edilen extract GC/MS de seçilmiş ion tarama (SIM) konumunda analiz edilmiştir. Bu yöntemde amfetamin için %93.6, metamfetamin için %94.1 verim değerleri hesaplanmıştır.*

Anahtar kelimeler: *Amfetamin, metamfetamin, GC/MS metodu*

INTRODUCTION

Illicit amphetamine and methamphetamine are largely derived by synthesis in clandestine laboratories. There is a marked variation in the occurrence of amphetamine and methamphetamine in different parts of the world. Amphetamine is more prevalent in Europe whereas methamphetamine is the more common drug in the USA, Japan and South-East Asia. In connection with their never-ending popularity and extensive use, the analytical procedures for determining amphetamine and methamphetamine in biological fluids have become more important in recent years[1].

Amphetamine is most frequently taken orally or intranasally as the sulfate or phosphate salt, while methamphetamine is most frequently prepared for injection or for smoking although is also available in tablet form (2,3). Following 2.5-15 mg, oral doses of Amphetamine peak plasma levels of 30-170 mg/l are reached in 2 hours and plasma elimination half lives range from 8 to 12 hours. Blood concentrations in fatalities are normally above 500 mg/l[2,3].

Amphetamine and methamphetamine begin to appear in the urine within 20 minutes of administration and both are excreted unchanged Typically 20-30% of the administrated dose of amphetamine is excreted unchanged. The recommended target analytes are, therefore, the unchanged drugs. Benzoic acid, hippuric acid and hydroxylated metabolites

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could be evaluated as the major metabolites of amphetamine[2,4,5,6]. Methamphetamine is excreted also as the unchanged drug over 44% of the administrated dose. Amphetamine and 4-OH-methamphetamine appear as the major metabolites of metamphetamine[2]. The metabolic pathways of amphetamine and methamphetamine are summarized in Figure 1.

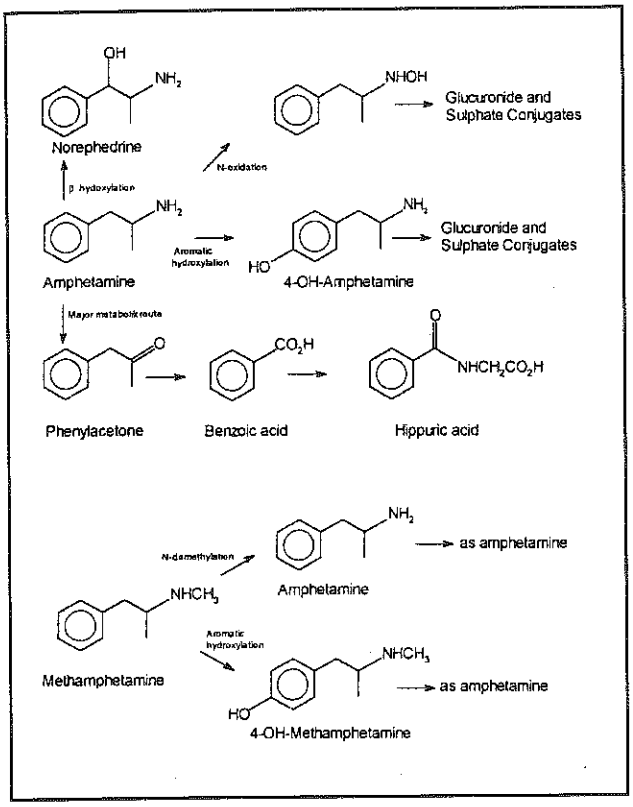

Figure 1. Metabolic pathways of amphetamine and methamphetamine.

Many studies have been carried out on the determination of amphetamine and methamphetamine in recent years[7,8,9]. The Gas chromatographic method (GC) is one of the most used procedures for analyzing amphetamine in biological fluids. A Gas Chromatographic method for determination of amphetamine in urine had been carried out in our department also in 1983 and amphetamine was detected by its N-acetyl derivatives by using FID detector[10]. In the present study we used TFA as a derivatization agent and the procedures were performed by using GC/MS in combination with solid-phase extraction. This combination provided extremely high recovery and reproducibility values.

MATERIALS AND METHODS

Chemicals and Reagents : Amphetamine, methamphetamine and their analog deuterated internal standards were supplied by Radian Corporation, Austin, TX. Amphetamine-D_5 and methamphetamine - D_5 were used as internal standards in GC/MS. Derivatization was carried out with Trifluoroacetic Anhydride (TFA) which was supplied by Pierce. Amphetamine and methamphetamine were extracted using Bond Elut Certify solid-phase extraction columns (Varian Sample Preparation Products, Harbor City, CA). The other chemicals were supplied by Merck.

The deuterated internal standards were obtained as solutions at concentrations of 100 µg/mL Stock deutered internal standard solutions were prepared by diluting to give a final concentration of 20 µg/mL. Amphetamine and methamphetamine standards were also obtained as solutions at concentrations of 1 mg/ml and were also diluted to give a concentration of 10 µg/ml. These final solutions were used as stock standard solutions.

0.1 M Phosphate Buffer, pH 6: 13.61 g of KH_2PO_4 was added to 900 mL deionized water and dissolved. The pH was adjusted to 6.0 with 1.0 M KOH and the total volume was brought up to 1.0 L with deionized water.

2% Ammonium Hydroxide in Ethyl Acetate: 2 mL of concentrated ammonium hydroxide was added to 98 mL of ethylacetate and shaken vigorously for 5 min.

Instrumentaton : Analyses were performed on a Hewlett Packard Model 5890 series II Gas Chromatograph and 5971 MSD with a 7673 autosampler and a 12 m x 0.2 mm x 0.33 µm film HP Ultra-1 capillary column. The other parameters were given below;

Column pressure: 8.5 psi, Septum purge flow: 0.8 mL/min, Split went flow: 20 mL/min, Column flow: 1.04 ml/min, Column temp. 1: 60°C (time: 0.5 min, increment: 20°C/min) Column temp. 2: 220°C (time: 0, increment: 40°C/min), Column temp. 3: 275°C (time: 2 min), Injector temp.: 250°C, interface temp.: 280°C, injection mode: splitless, Dwell Time (DT): 100 ms.

Ions monitored were: amphetamine-TFA m/z 140, 118, 91; methamphetamine-TFA m/z 154, 118, 110; amphetamine-D_5-TFA m/z 144, 123, 58; methamphetamine-D_5-TFA m/z 158, 113. For quantification the ion ratios were m/z 140/144 for amphetamine and m/z 154/158 for methamphetamine (The bold ion numbers were used for quantification).

Preparation of standards: 0.5 µg/ml, 1.0 µg/mL, 1.5 µg/ml of amphetamine and methamphetamine standards were prepared in 2 ml of drug free urine. All the standards were extracted and derivatized as described in the axtraction and derivatization procedure below.

Extraction procedure : 2 ml of urine (prepared above) and 2 ml 0.1 M phosphate buffer (pH: 6.0) were added to a suitable test tube. The solution was vortexed and poured on an SPE column, previously activated by the sequential addition and elution of 2 mL of methanol and 2 mL of 0.1 M phosphate buffer (pH:6.0). The buffered urine was eluted with vacuum. The column was washed by the addition and elution of 1 mL 1.0 M acetic acid and dried under maximum vacuum for 2 min. The analytes were eluted into a 5 ml screw capped tube by adding 2 mL of freshly prepared 2% ammonium hydroxide in ethylacetate. The eluent was fully evaporated at 30-40°C under a slow flow of nitrogen after 20 µl of methanol with 1% of HCl had been added to the eluent to prevent evaporation of the amphetamines[2,11].

Derivatization: The residue of the evaporated solution was reconstituted with 200 µl of TFA. The tube was capped, vortexed and placed in a heating block at 70°C for 30 min. After cooling, 1 µl of the derivatized solution was injected into the GC/MS[2,12].

Linearity : 100, 200 and 300 µl of each analyte were added to the drug free urine (as described in preparation of standards) and the linearity of the extracted amphetamine and methamphetamine determined. These urine samples were extracted and derivatized in the described manner. The ion ratios for quantitating each analyte were plotted against their respective concentrations. The plots were subjected to linear regression analyses (Fig 4).

Recovery Studies : Drug-free urine was used to prepare standard solutions of 1 µg/ml of amphetamine and methamphetamine. These standard solutions were extracted in the previously described manner, with the exception that no internal standards were added to the urine prior to extraction. After extraction and prior to derivatization, 20 µl of deuterated internal standards were added to the extracts. The derivatized extracts were analyzed under the described conditions. The area ratio of the extracted drugs to their respective internal standards was compared to the corresponding area ratio of unextracted 1 µg amphetamine and methamphetamine standards. The recovery results are the mean of three determinations (Table 1).

Reproducibility studies : Reproducibilities of the analyses were determined by calculating coefficients of variation. Both amphetamines (0.1 / 0.3 µg/mL) and methamphetamine (0.1/0.3 µg/mL) containing urine standards was analyzed in 10 separate runs Table 2).

RESULTS AND DISCUSSION

In this study the solid-phase extraction (SPE) and the TFA derivatization techniques were used simultaneously in combination with the GC/MS method. This combination is very rapid and provided an excellent linearity, recovery and reproducibility for routine laboratory use. The limits of detection for amphetamine and methamphetamine were calculated as 15 ng/mL at our working conditions[13].

Derivatization is one of the most commonly used technique for increasing the volatility and thermal stability of the molecule[12]. The character of the molecule is changed from active to inert. Thus the interactions between the column support and the derivatized form of the analyte are reduced to a minimum and the chromatograms exhibit well-resolved Gaussian-shaped peaks with no observable interferences[12,14]. Selected Ion Monitoring (SIM) chromatograms and mass spectra of the trifluoroacethyl derivatives (TFA) of standards and internal standards are shown in Fig. 2,3.

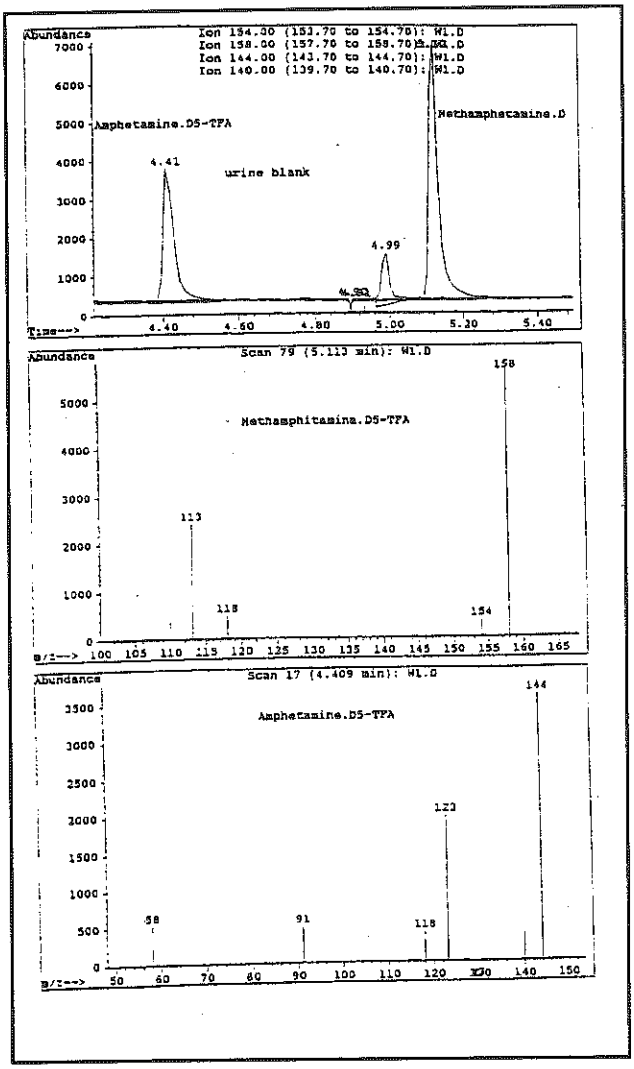

Figure 2. SIM chromatogram and mass spectra for trifluoroacetyl derivatives of internal standards (amphetamine-D_5, and metamphetamine-D_5 200 ng/mL) in 2 mL of urine.

The analytes were derivatised to improve their chromatographic properties and to provide higher mass ions for measurement than underivatized analytes give (interference is lower at higher masses). The commonly used derivatives are pentafluoropropionyl, heptafluorobutyryl (HFB), N-trifluoroacetyl-1-prolyl chloride (TPC) and trifluoroacetyl (TFA). Although some publications were made in the past concerning the lack of stability of trifluoroacetyl derivatives, TFA has been used successfully in a number of studies in recent years as a derivatization agent[12,14]. In our study we observed that with use of deuterated internal standards, TFA derivatives were quantitatively stable

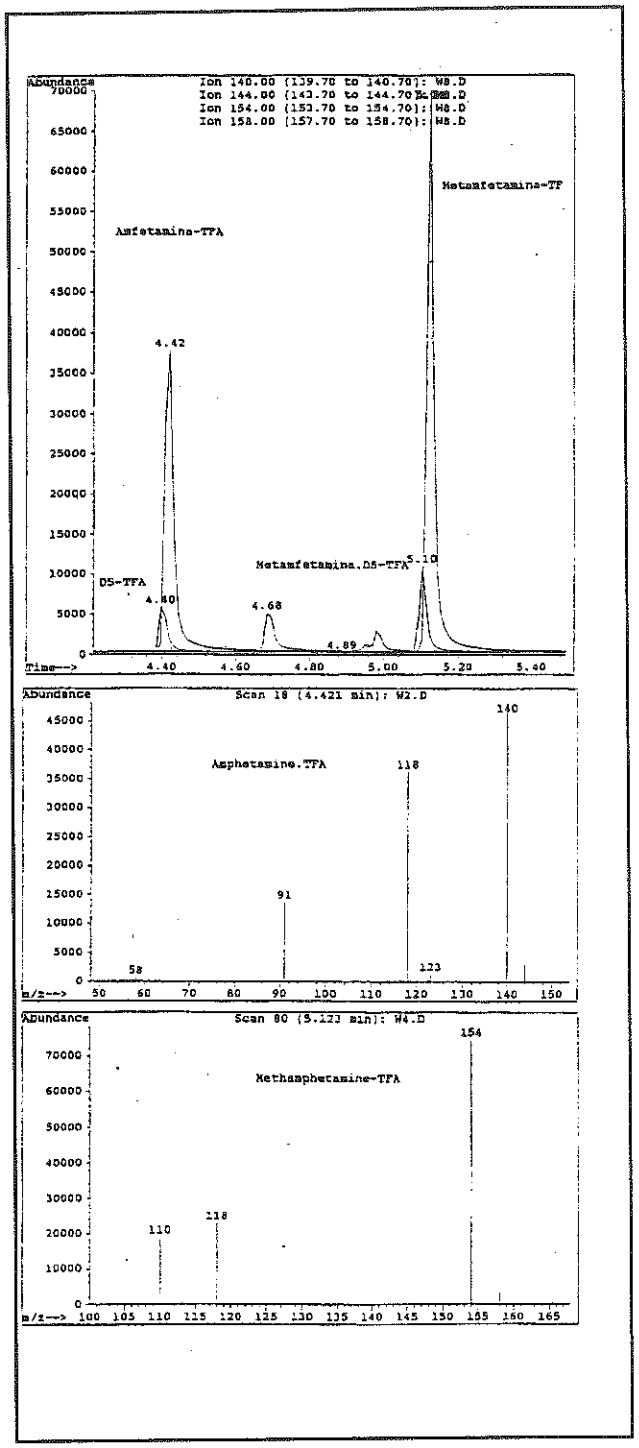

Figure 3. SIM chromatogram and mass spectra for trifluoroacetyl derivatives of standards (amphetamine and metamphetamine, 1 µg/mL) in 2 mL of urine. Deuterated analogs of trifluoroacetyl derivatives of internal standards can also be seen in SIM chromatogram.

for at least one day. Fuller et al. (1992) performed a similar study on codeine and morphine by using TFA as the derivatization agent and found that the trifluoroacetyl derivatives were stable over a period of 2 to 3 days. A similar study was performed by Ellerbe et al. (1993) and the stability of trifluoroacetyl derivatives of amphetamine and methamphetamine was also found to be 2 days. Our observations were in agreement with both of the studies.

Linearity : As shown in Figure 4, the "R^2" values of amphetamine and methamphetamine were calculated as 0.9999 an 0.9974 respectively. The y-intercepts were -0.0148 for amphetamine and -0.0804 for methamphetamine. These results provided us with and adequate quantification in our working range (Fig 4).

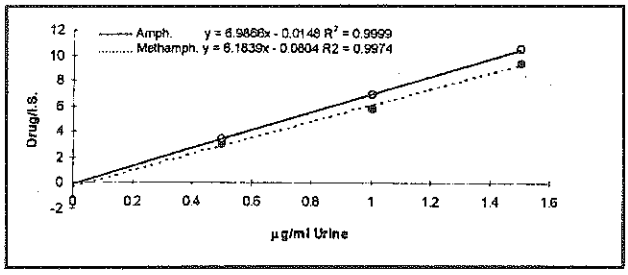

Figure 4. Quantitative ion ratios versus concentrations of drug in spiked urine.

Recovery : The recovery rates for ampehatimine and methamphetaine were shown in Table 1. Jonsson et all (1996) and Tatsuno et all (1996) reported 83% and 88% recovery rates respectively in their studies. Our extraction procedure provides relatively higher recovery rates than these studies (15,16).

Table 1. The results of the recovery studies

	Recovery (%)	
	Mean*	Range
Ampehatamine	93.6	92.9 - 94.2
Methamphetamine	94.1	93.2 - 95.7

* The results are the mean of three determinations (1 µg/ml)

Reproducibility : An adequate reproducibility was provided by this method. The coefficient of variation (CV) for amphetamine and methampehetaine can be seen in Table 2.

Table 2. The results of the reproducibility studies

	Reproducibility							
Drugs	Actual		Found*		S.D.		C.V.	
	µg/ml		µg/ml		µg/ml			
Amph.	0.1	0.3	0.103 (0.096-0.115)	0.305 (0.291-0.316)	0.005	0.007	4.9	2.4
Methamph	0.1	0.3	0.101 (0.094-0.116)	0.303 (0.285-0.319)	0.005	0.0011	5.1	3.6

* The concetrations are the mean of ten determinations

Solid-phase extraction, which is emerging as a very important sample preparation technique, was preferred to other traditional extraction procedures, such as liquid-liquid extraction (LLE). Higher recoveries of the analytes, higher selectivity and highly purified extracts, reduction in organic solvent consumption and time were observed as the advantages of SPE procedure over liquid/liquid extraction[17,18]. Bound Elute Certify®, which we used in this study, represents a copolymeric bonded silica column. Depending on the elution fluid and pH condition these copolimeric bonded silica sorbents, have three different types of interactions: hydrophobic, polar and ionic. These interactions provide the selectivity, resulting in extremely clean extracts leading to an improved precision of analysis[17,18].

In this method an extraction (SPE method) and derivatization procedure (TFA) of amphetamine and methamphetamine in urine was described. This combination was used several times to determine urinary amphetamine and methamphetamine in many earlier studies. However some modifications, especially in eluting and derivatization procedures, were made in our study. These modifications provided us with better recovery rates when we compared these with the earlier studies[14,15]. Consequently when the linearity, recovery and reproducibility values that we obtained from our study were taken into consideration, this method can be recommended for routine laboratory use.

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