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RAPID AND HIGHLY SENSITIVE METHOD FOR DETERMINATION OF METHAMPHETAMINE AND AMPHETAMINE IN URINE BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY

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SUMMARY

A rapid and sensitive method for determination of methamphetamine and amphetamine in urine was developed by using electron-capture gas chromatography. The extraction procedure, the experimental conditions for pentafluorobenzoyl derivative formation and the percentage recovery of the drugs from urine are described.

The pentafluorobenzoyl derivative of methamphetamine showed a higher electron-capture sensitivity and was detected in at least 23-fold lower concentration than the heptafluorobutyryl derivative which is commonly used as a derivatizing agent for the amine. The detection limit of pentafluorobenzoyl derivatives of methamphetamine and amphetamine was *ca.* 10 pg. A concentration as low as 10 ng/ml of methamphetamine and amphetamine in urine was easily detected by this method.

INTRODUCTION

Various methods for the identification of methamphetamine and other related amines in biological material have been presented during recent years. These methods include gas chromatography (GC)[1–6], thin-layer chromatography (TLC)[4–7], spectrophotofluorometry[8,9], high-performance liquid chromatography (HPLC)[10,11], gas chromatography–mass spectrometry (GC–MS)[12–14] and immunoassay[11,15–17].

In recent years, the number of abusers of methamphetamine has been markedly increasing again in Japan. Thus, the development of a more simple, rapid and sensitive assay method for methamphetamine has been required.

Using the electron-capture detector (ECD) it has become feasible to analyse

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methamphetamine and related amines at highly sensitive levels. In this paper, we report a more sensitive derivatization of methamphetamine and amphetamine for the ECD, and the experimental conditions for determination of these amines in urine.

EXPERIMENTAL

Apparatus

GC analysis was carried out with a Hitachi 023 gas chromatograph equipped with a ^{63}Ni ECD. A glass column (2 m × 2 mm I.D.), packed with 2% Thermon-3000 on Chromosorb W (AW-DMCS), 80–100 mesh, was used. The temperatures were: oven, 200°C; injection port, 220°C; detector, 200°C. The nitrogen flow-rate was 50 ml/min. The chart speed was 5 mm/min. The mass spectrometric analysis was carried out on a JEOL JMS-D300 mass spectrometer connected to the chromatograph. A glass column (2 m × 2 mm I.D.) packed with 3% OV-17 on Gas-Chrom Q, 80–100 mesh, was used. The temperatures were: oven, 260°C; injection port, 280°C; separator, 250°C; ion source, 240°C. The ion current was 300 μA and the ion voltage 300 eV. Methane was used as reactant gas.

Reagents

Trifluoroacetic, pentafluoropropionic and heptafluorobutyric anhydrides and pentafluorobenzoyl chloride were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Acetone (specially prepared reagent) and *n*-pentane were obtained from Nakarai and Wako (Osaka, Japan), respectively. These solvents were redistilled and then used in these experiments. Methamphetamine hydrochloride was obtained from Dainippon Seiyaku. All other chemicals were of reagent grade.

Extraction of methamphetamine and amphetamine from urine

To diluted urine (0.2 → 2.0) in a 10-ml stoppered round-bottomed centrifuge tube were added 0.5 ml of 10 *M* NaOH and 6.0 ml of *n*-pentane. The contents were mixed for 10 min and centrifuged for 5 min at 800 *g*. Next, 5 ml of the *n*-pentane layer were transferred to a 10-ml screw-cap round-bottomed centrifuge tube, and 1 ml of 400 nmol/ml pentafluorobenzoyl chloride, dissolved in *n*-pentane, was added to the extract and incubated at 80°C for 20 min. Then the mixture was cooled and washed twice with 2.0 ml of 0.1 *M* $NaHCO_3$ and once with 2.0 ml of distilled water. Following centrifugation for 5 min at 800 *g*, 5.0 ml of the *n*-pentane layer was transferred to a 10-ml stoppered conical-bottomed centrifuge tube, and a small antibubbling granule was added. The sample was evaporated at 50°C until just dry. The residue was dissolved in acetone and an aliquot (1–3 μl) was injected into the gas chromatograph.

Electron-capture response of various methamphetamine derivatives

A mixture of 1.0 ml of methamphetamine (10 μg/ml in *n*-pentane) and 4.0 ml of *n*-pentane was transferred to a 10-ml screw-cap round-bottomed centrifuge tube, to which was added 0.2 ml of heptafluorobutyric anhydride or 0.2 ml of pentafluoropropionic anhydride or 0.5 ml of trifluoroacetic anhydride or 0.2 ml of pentafluorobenzoyl chloride (20 μmol/ml in *n*-pentane). The tubes were incubated at 65°C for 20 min for trifluoroacylation or 60 min for formation of the other derivatives. The tubes were

cooled to room temperature. The *n*-pentane layer was washed twice with 2.0 ml of 0.1 *M* $NaHCO_3$ and once with 1.0 ml of distilled water. The *n*-pentane layer was transferred to a 10-ml stoppered conical-bottomed centrifuge tube and evaporated under nitrogen. The residue was dissolved in acetone and subjected to GC–ECD.

Preparation of pentafluorobenzoyl derivative of methamphetamine

A mixture of 5.0 ml of methamphetamine solution (10 mg/ml in *n*-pentane), 0.8 ml of pentafluorobenzoyl chloride and 50 μl of pyridine was warmed at 65°C for 90 min. The mixture was cooled and washed six times with 1 ml of 10 *M* NaOH and twice with 5.0 ml of distilled water. The *n*-pentane layer was evaporated at room temperature. The crystalline layer was recrysrtallized from *n*-pentane and analysed by a melting point determination and GC–MS. The crystals were dissolved in acetone and used as an authentic standard of the pentafluorobenzoyl derivative of methamphetamine for GC.

RESULTS AND DISCUSSION

Mass spectrum of authentic standard of pentafluorobenzoyl derivative of methamphetamine

The crystals (m.p. 96–97°C) obtained from methamphetamine and pentafluorobenzoyl chloride were white and scaly, and their chemical ionization mass spectrum is shown in Fig. 1. The parent ion of the pentafluorobenzoyl derivative of methamphetamine (MW 343) yielded the following *m/z* fragments, 344 (QM^+, M + 1) and 372 $(M + C_2H_5)^+$. From the resulting spectrum, the crystals were identified as the pentafluorobenzoyl derivative of methamphetamine, and thereafter used as an authentic standard.

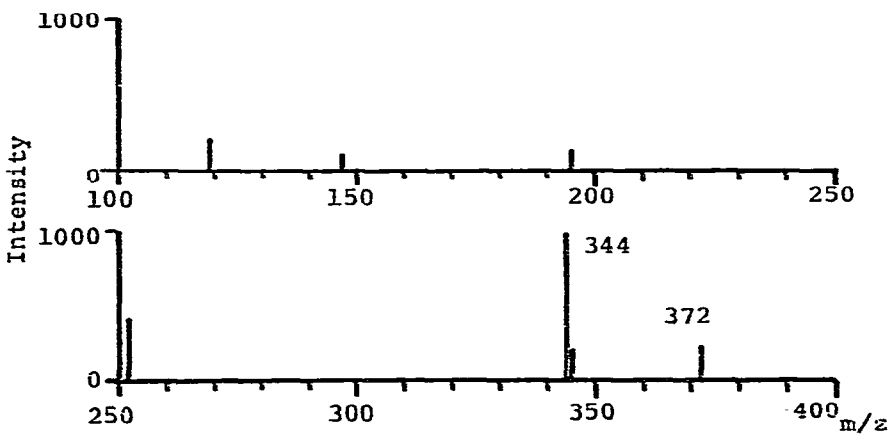

Fig. 1. Chemical ionization mass spectrum of the pentafluorobenzoyl derivative of methamphetamine.

Electron-capture response of various methamphetamine derivatives

Experiments were carried out to compare the electron-capture affinity of the various derivatives of methamphetamine, including the pentafluorobenzoyl derivative of the amine. As shown in Table I, the electron-capture affinity of the penta-

TABLE I

RELATIVE SENSITIVITIES OF VARIOUS METHAMPHETAMINE DERIVATIVES

Derivative	*Sensitivity**
Trifluoroacetyl	0.028
Pentafluoropropionyl	3.19
Heptafluorobutyryl	24.25
Pentafluorobenzoyl	560.00

* Peak height mm/ng methamphetamine.

fluorobenzoyl derivative of methamphetamine was 23 times and 176 times higher than that of the heptafluorobutyryl and pentafluoropropionyl derivatives, respectively. The results indicate that the pentafluorobenzoyl derivative of methamphetamine showed a higher affinity towards the ECD than other derivatives of the amine.

In accordance with the present findings, Wilkinson and Beckett[18] have reported that the pentafluorobenzoyl derivative of phenylalkylamine has a greater electron-capture affinity. Midha *et al.*[19] have also reported that the electron-capture affinity of the pentafluorobenzoyl derivative of ephedrine is higher than those of the heptafluorobutyryl, pentafluoropropionyl and trifluoroacetyl derivatives.

Because the pentafluorobenzoyl derivative of methamphetamine showed a higher electron-capture affinity and thus seemed to be a good derivatizing agent for the determination of the amine by GC–ECD, we examined the detailed experimental conditions for the derivatization of the amine as described below.

Effect of amounts of pentafluorobenzoyl chloride on methamphetamine derivatization

To a mixture of 1.0 ml of methamphetamine (100 ng/ml in *n*-pentane) and 4.0 ml of *n*-pentane was added 1.0 ml of 500, 100, 20, 3, 0.8 or 0.16 nmol/ml pentafluorobenzoyl chloride dissolved in *n*-pentane. The rates of formation of pentafluorobenzamide were calculated from peak heights of the authentic standard of pentafluorobenzoyl derivative of methamphetamine (100 pg).

TABLE II

EFFECT OF PENTAFLUOROBENZOYL CHLORIDE (PFB-Cl) AMOUNTS ON FORMATION OF PFB DERIVATIVE OF METHAMPHETAMINE

Methamphetamine, 100 ng (0.65 nmol); reaction time, 20 min; temperature, 80°C.

PFB-Cl (nmol)	*Formation of PFB-methamphetamine (%)*
500	102.0
100	105.0
20	100.0
4	97.6
0.8	68.6
0.16	30.7

As shown in Table II, when 4 nmol or more of pentafluorobenzoyl chloride was added to the reaction mixture, there was complete formation of pentafluorobenzamide. Thereafter, we used 400 nmol of pentafluorobenzoyl chloride to derivatize methamphetamine.

Effect of reaction time and temperature on methamphetamine derivatization

A mixture of 1.0 ml of methamphetamine standard solution (100 ng in *n*-pentane), 4.0 ml of *n*-pentane and 1.0 ml of pentafluorobenzoyl chloride (4 nmol/ml in *n*-pentane) was warmed at 40°C, 60°C or 80°C for 10, 20, 40, 60 or 90 min.

As shown in Fig. 2, derivatization of methamphetamine was complete within 20 min. Variation of the reaction temperature did not show any appreciable effect on the formation of pentafluorobenzamide under the experimental conditions. Based on these results, the experimental conditions for the derivatization of methamphetamine were chosen as follows: reaction time, 20 min; temperature, 80°C.

The calibration curves for methamphetamine and amphetamine obtained under the experimental conditions are shown in Fig. 3. There was a good linearity between peak height and methamphetamine or amphetamine amount ranging from 10 to 150 pg.

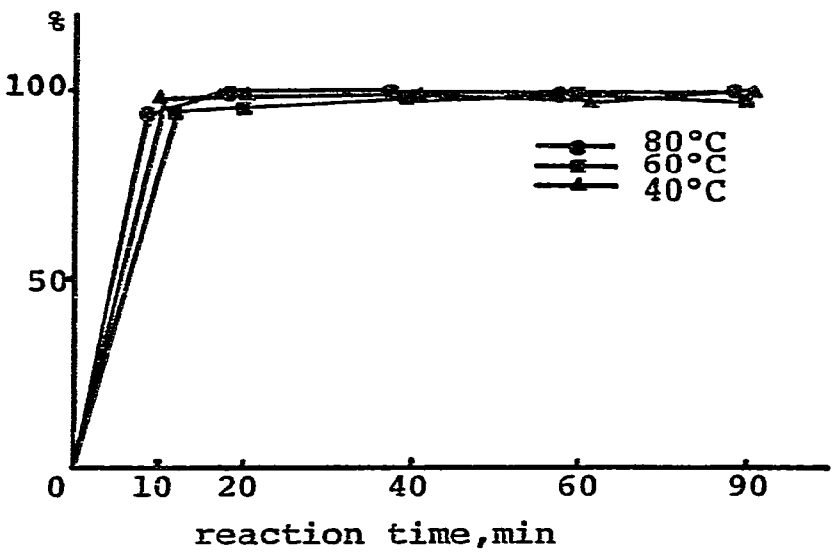

Fig. 2. Effect of reaction time on the formation of the pentafluorobenzoyl derivative of methamphetamine.

Gas chromatograms

Because the pentafluorobenzoyl derivative of methamphetamine is highly sensitive to the ECD, we applied this method to biological material.

Gas chromatograms of pentafluorobenzoyl derivatives of methamphetamine and amphetamine from urine are shown in Fig. 4. Fig. 4A shows a typical gas chromatogram obtained from extracts of blank urine. No extraneous peaks are observed. Fig. 4B shows a gas chromatogram obtained from a standard 100-pg pentafluorobenzoyl derivative of methamphetamine and amphetamine, and Fig. 4C shows a gas chromatogram from an extract of urine containing methamphetamine and amphetamine (50 ng/ml).

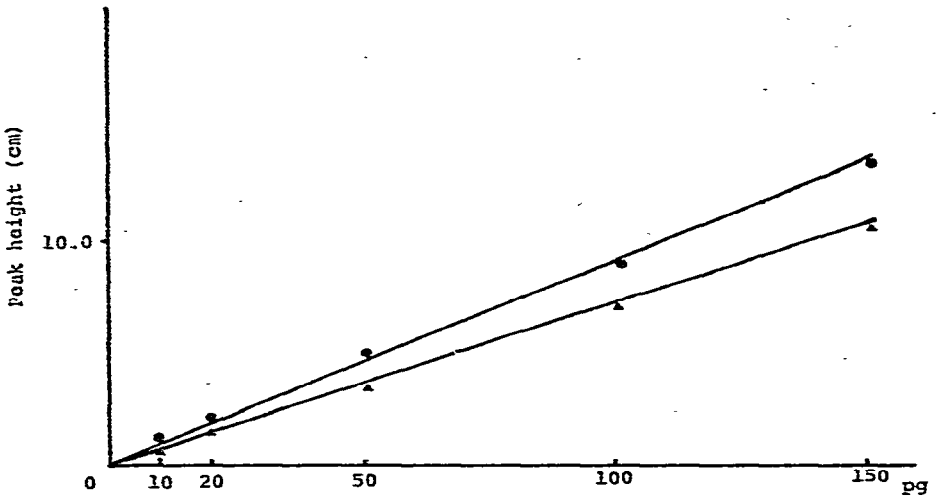

Fig. 3. Calibration curves of the pentafluorobenzoyl derivatives of methamphetamine (●) and amphetamine (▲).

Retention times of the pentafluorobenzoyl derivatives of methamphetamine and amphetamine using various packing materials are listed in Table III. When Thermon-3000 was used as packing material, the pentafluorobenzoyl derivatives of methamphetamine and amphetamine were separated clearly and had retention times of 3.3 min and 6.9 min, respectively. Separation of the pentafluorobenzoyl derivatives

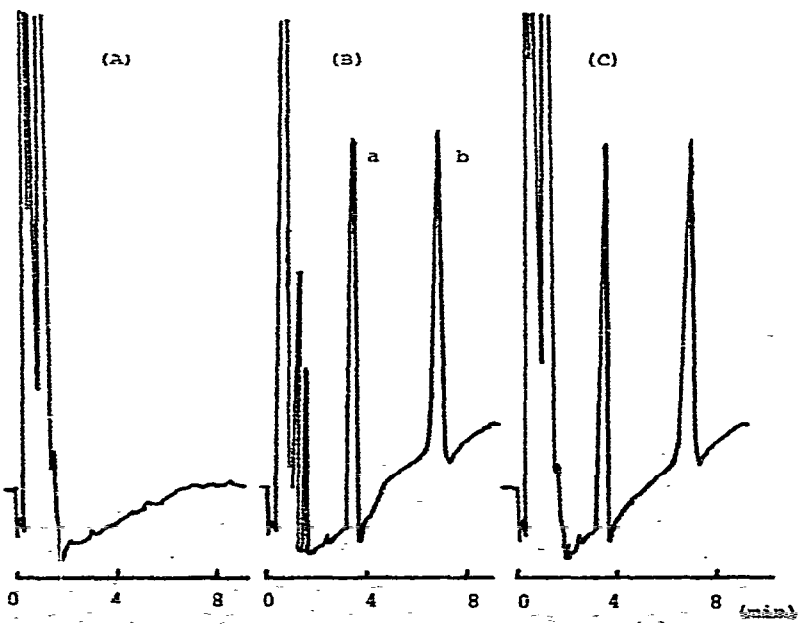

Fig. 4. Gas chromatograms of (A) extract from blank urine, (B) 100 pg of pentafluorobenzoyl chlorides of methamphetamine (a) and amphetamine (b), and (C) extract from urine containing 50 ng/ml methamphetamine and amphetamine. Retention times for (a) and (b) were 3.3 and 6.9 min, respectively.

TABLE III

RETENTION TIMES OF PENTAFLUOROBENZOYL DERIVATIVES OF METHAMPHETAMINE AND AMPHETAMINE

Compound	*Retention time (min)*			
	3% QF-1, 2 m, 200°C	*1.5% SE-30, 2 m, 200°C*	*2% OV-17, 3 m, 220°C*	*2% Thermon-3000, 2 m, 200°C*
Methamphetamine	6.92	5.01	6.98	3.32
Amphetamine	6.65	4.72	6.95	6.86

TABLE IV

RECOVERIES OF METHAMPHETAMINE AND AMPHETAMINE FROM URINE

Methamphetamine and amphetamine added to 0.2 ml of urine	*Recovery (%)**	
	Methamphetamine	*Amphetamine*
5 ng	107.1 ± 0.05	100.2 ± 0.06
50 ng	93.9 ± 0.85	98.7 ± 0.60

* Mean ± standard error.

of methamphetamine and amphetamine was incomplete when OV-17 or OV-225 was used as the packing material.

Recovery from urine

The results of the recovery experiments of methamphetamine and amphetamine, added to urine, are shown in Table IV. The average percentage recoveries for methamphetamine and amphetamine were 93.9–107.1 and 98.7–100.2, respectively. Because the recoveries of both methamphetamine and amphetamine when added to urine were satisfactory, the assay method presented would be applicable for the determination of these amines in biological materials.

Bruce and Maynard[20] have reported the determination of heptafluorobutyryl derivatives of amphetamine and related amines in blood by GC–ECD. The detection limit of methamphetamine and amphetamine by their method was 400 pg. Driscoll *et al.*[21] used the trichloroacetamide derivative for the determination of methamphetamine, with a detection limit of 25 pg. They reported that the derivative of methamphetamine showed a greater sensitivity than those of heptafluorobutyramide and pentafluorobenzamide.

In the present study, the detection limit of the pentafluorobenzoyl derivatives of methamphetamine and amphetamine was shown to be *ca.* 10 pg. This indicates that a concentration as low as 10 ng/ml of methamphetamine or amphetamine in urine can easily be detected by this procedure.

Matin and Rowland[22] have shown that the order of electron-capture sensitivity for primary amines is as follows; pentafluorobenzamide > pentafluorobenzylidine > heptafluorobutyramide, and that primary amines exhibit a greater sensitivity

than secondary amines. In the present study, however, it was found that the sensitivities of pentafluorobenzoyl derivatives of methamphetamine and amphetamine were similar.

In conclusion, GC–ECD of both methamphetamine and amphetamine, after derivatization of the amines with pentafluorobenzoyl chloride, was rapid and highly sensitive and would be suitable for the determination of these amines in biological materials.

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