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Rapid and simple determination of methamphetamine and amphetamine in blood by simultaneous extraction–derivatization

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Abstract

A method capable of extracting and derivatizing methamphetamine (MA) and amphetamine (AM) in blood simultaneously was developed. In this method ethyl acetate, triethylamine and pentafluorobenzyl bromide were added to blood, the extraction and pentafluorobenzyl derivatization of MA and AM were performed simultaneously by heating and stirring on a hot plate stirrer. Pentafluorobenzyl derivatives were determined by gas chromatography/chemical ionization-mass spectrometry (GC/CI-MS). Deuterium labeled MA and AM were used as internal standards. This method enabled an accurate and precise quantitative analysis from blood containing MA and AM. Excellent results were obtained for autopsy cases with possible MA. © 1997 Elsevier Science B.V.

Keywords: Methamphetamine; Amphetamine; Pentafluorobenzyl derivatives; Gas chromatography/mass spectrometry; Forensic toxicology

1. Introduction

The abuse of methamphetamine is still increasing and rapid and simple determinations of methamphetamine and amphetamine, its principal metabolite, in blood has been required in forensic and clinical toxicology. For quantification of MA and AM in human fluids, gas chromatography (GC) with electron capture detector [1–3], flame thermoionic detector [4] or nitrogen-phosphorus detector [5], high-performance liquid chromatography (HPLC) with ultraviolet detector [6] and chemiluminescence detector [7,8] and gas chromatography/mass spectrometry (GC/MS) by selected ion monitoring (SIM) [9–11] have been previously reported. In these procedures, GC/MS has been most widely used owing to its high sensitivity and selectivity.

MA and AM generally required derivatization of the amino group prior to GC/MS analysis in order to improve their chromatographic properties and obtain a higher mass fragmentation pattern. Derivatives used in previous methods include trifluoroacetyl [4,9,10,12], trichloroacetyl [1], heptafluorobutyryl [2], pentafluorobenzoyl [3], 4-carbethoxyhexafluorobutyryl [11] and *n*-propyl

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[5]. Most of these derivative agents can not react in the presence of aqueous solution. Therefore, after liquid–liquid or solid phase extraction the derivatization is generally carried out in an organic solvent. The object of this study was to develop an analytical procedure that is rapid and simple by combining extraction and derivatization using a derivatization agent that could react in the presence of aqueous solution.

In this report, we described the rapid and simple determination of MA and AM using pentafluorobenzyl bromide (PFBBr). Extraction and derivatization were simultaneously conducted in the presence of ethyl acetate by stirring on a hot plate stirrer. PFB derivatives of MA and AM were determined by GC/CI-MS. This method was applied to blood from autopsy cases with possible MA toxicity, which is useful for forensic and therapeutic toxicology.

2. Experimental

2.1. Reagents

AM sulfate was prepared according to the method of Ramirez and Burger [13] and MA hydrochloride was purchased from Dainippon Pharmaceuticals Co. Ltd., Osaka. MA-d_5 hydrochloride and AM-d_5 sulfate were prepared according to the method of Sano et al. [9]. This method which utilizes the replacement of hydrogen atoms at the benzene ring with deuterium atoms was adopted because it is an easy procedure without organic synthesis. The purities of MA-d_5 and AM-d_5 were 72% [MA-d_4 10, MA-d_3 15, MA-d_2 3%] and 85% [AM-d_4 5, AM-d_3 10%], respectively. PFBBr was purchased from Aldrich Japan Inc. and other common chemicals used were of the highest purity commercially available.

2.2. Apparatus

Extraction and derivatization were performed using a hot plate stirrer, Advantec SR350 (Advantec, Tokyo). GC/MS analyses were carried out with a JMS-DX 300 MS equipped with an MS-GCG 05 gas chromatograph (JEOL, Tokyo). GC was carried out with a DB-1 wide bore capillary column (15 m $\times$ 0.53 mm i.d., 1.50 μm film thickness, J&W, California). The GC conditions were: column temperature 155°C, injection temperature 200°C and helium flow rate 30 ml min^{-1}. The CI-MS conditions were: regent gas methane, ionization voltage 200 V, ionization current 300 μA, separator and inlet temperature 200°C and chamber temperature 200°C. Molecular protonated ions at m/z 330, 316, 335 and 321 were monitored for PFB-MA, PFB-AM, PFB-MA-d_5 and PFB-AM-d_5, respectively.

2.3. Extraction and derivatization

Whole blood (1 ml) was put in a 20 ml Erlenmeyer flask and an aqueous solution (2 ml) containing 3.82 μg MA-d_5 and 3.13 μg AM-d_5 as internal standard was added. The solution was then buffered at pH 9.0 with 0.5 ml of 0.1 M boric acid-sodium hydroxide buffer and ethyl acetate (5 ml), triethylamine (30 μl) and PFBBr (30 μl) were added in turn. After the flask was capped the mixture was stirred and heated on a hot plate stirrer for 30 min and extraction and pentafluorobenzyl derivatization were performed simultaneously. The temperature of the aqueous solution was set at 60 ± 3°C. Following centrifugation for 5 min at $1800 \times g$ the ethyl acetate layer was transferred to another tube and evaporated at 70°C under a stream of nitrogen. The residue was taken up in 200 μl of ethyl acetate and an aliquot (1 μl) was used for GC/MS analysis.

For standard samples, to 200 μl ethyl acetate containing MA, AM, MA-d_5, AM-d_5, 30 μl triethylamine and 30 μl PFBBr were added and heated at 60°C for 30 min with occasional mixing. Ethyl acetate was evaporated at 70°C under a stream of nitrogen and the residue was dissolved in 200 μl of ethyl acetate. The calibration curves for MA and AM were prepared with peak area ratios of MA and IS, AM and IS, respectively.

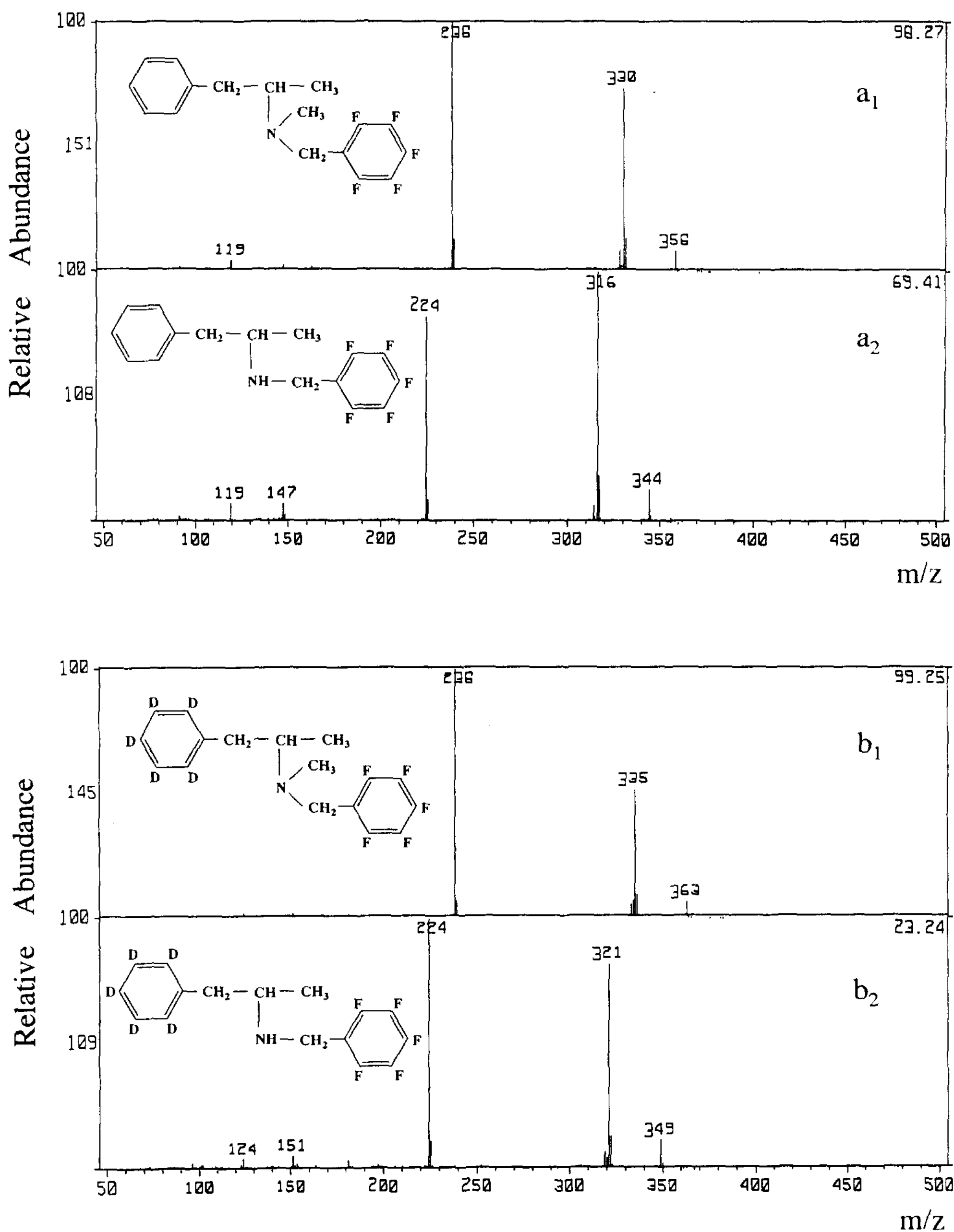

Fig. 1. CI mass spectra of unlabeled MA (a_1) and AM (a_2) and those of deuterium-labeled MA (b_1) and AM (b_2). All of them were measured as PFB derivatives.

The conditions determined as optimum for extraction–derivatization were examined by varying individual factors in the above procedure one by one. In this case pentafluorobenzyl derivatizations of MA-d_5 (3.82 μg) and AM-d_5 (3.13 μg), internal standards, were added after the extraction–

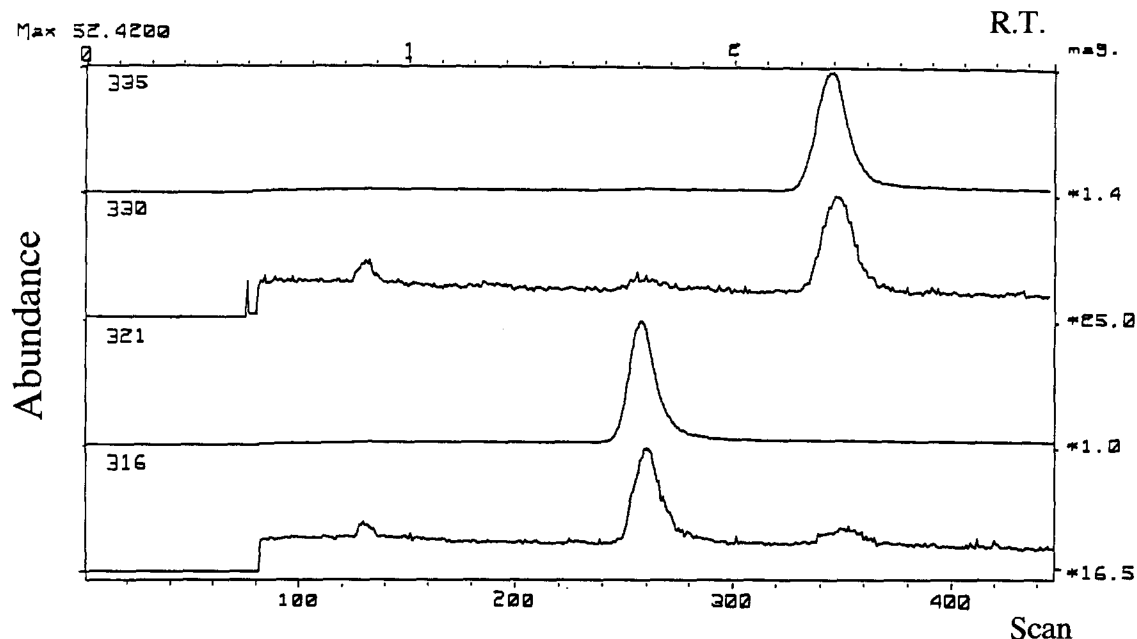

Fig. 2. SIM chromatogram for 1 ml of blood spiked with MA (103 ng) and AM (97 ng).The chromatograms on channels 316, 321, 330 and 335 correspond to AM, AM-d_5, MA and MA-d_5, respectively.

derivatization of MA (10.31 μg) and AM (9.38 μg). The relative intensity was determined by comparison with a standard sample.

3. Results and discussion

3.1. Mass spectra of PFB derivatives and SIM chromatogram

Chemical ionization mass spectra of PFB-MA (a_1), PFB-AM (a_2), PFB-MA-d_5 (b_1) and PFB-AM-d_5 (b_2) are shown in Fig. 1. The PFB-MA and PFB-AM yield very abundant $[M+1]^+$ peaks at m/z 330 and 316, respectively. For PFB-MA-d_5 and PFB-AM-d_5, the ions at m/z 335 and 321 were $[M+1]^+$ peaks and very abundant, respectively. The $[M+1]^+$ ions of labeled and unlabeled compounds did not overlap each other and no interferences were observed from blood constituents on any ion channel. Therefore, the four $[M+1]^+$ ions were monitored by GC/MS using SIM.A SIM chromatogram obtained from 1 ml of blood spiked with MA (103 ng) and AM (97 ng) is shown in Fig. 2. The retention time of PFB-AM and PFB-AM-d_5 was about 1.7 min and that of PFB-MA and PFB-MA-d_5 was about 2.3 min.

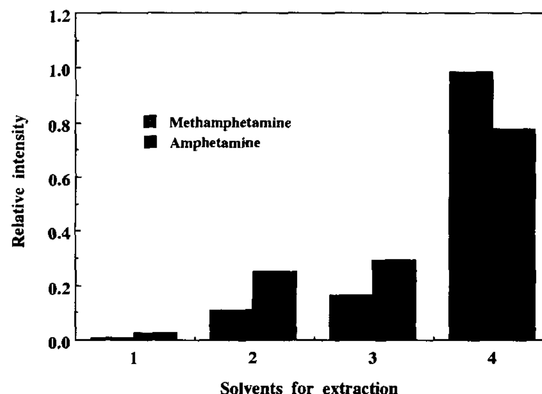

Fig. 3. Effect of solvents on extraction and derivatization of MA and AM. Solvents: 1, n-hexane; 2, toluene; 3, benzene; 4, ethyl acetate. 1 ml Blood spiked with MA (10.31 μg) and AM (9.38 μg) was determined. The longitudinal axis shows relative intensities to standard samples.

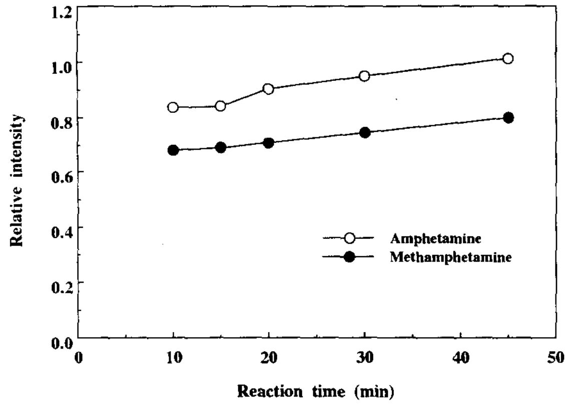

Fig. 4. Effect of heating time on extraction and derivatization of MA and AM. 10 ml Blood spiked with MA (10.31 µg) and AM (9.38 µg) was determined. The longitudinal axis shows relative intensities to standard samples.

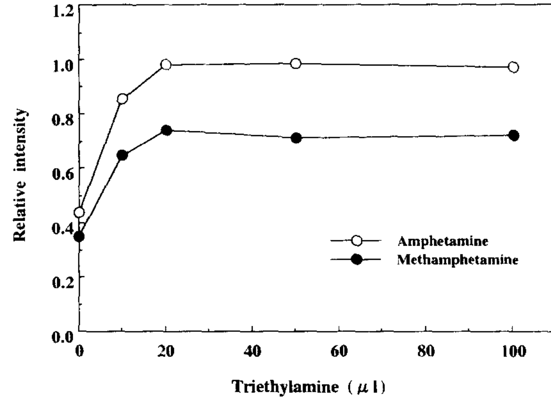

Fig. 6. Effect of added amout of triethylamine on extraction and derivatization of MA and AM. 1 ml Blood spiked with MA (10.31 µg) and AM (9.38 µg) was determined. The longitudinal axis shows relative intensities to standard samples.

3.2. Solvent for extraction and derivatization

The extraction and derivatization were carried out using four solvents, namely *n*-hexane, toluene, benzene and ethyl acetate. Because of the need to heat for derivatization, solvents with boiling points above 60°C were selected. PFB derivatives of MA and AM were only slightly detected for hexane as shown in Fig. 3 and were strongest for ethyl acetate. Ethyl acetate was therefore selected as the solvent for extraction and derivatization.

3.3. Reaction time and temperature

Derivatization increased only slightly between 10 and 45 min and so 30 min was selected (Fig. 4). As shown in Fig. 5, the production of PFB derivatives was accelerated by heating. However a temperature above 60°C was not practical because of the boiling point of ethyl acetate. Consequently 60°C, the maximum of feasible temperature, was selected.

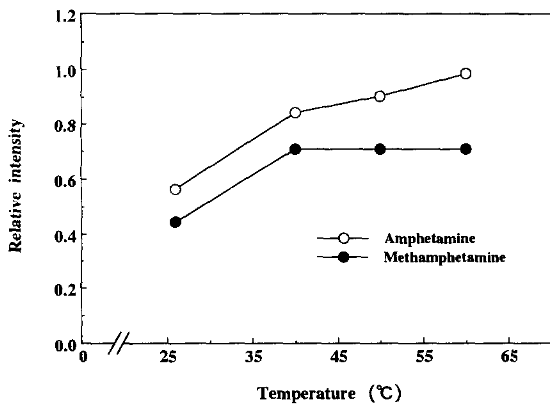

Fig. 5. Effects of heating temperature on extraction and derivatization of MA and AM. 1ml Blood spiked with MA (10.31 µg) and AM (9.38 µg) was determined. The longitudinal axis shows relative intensisites to standard samples.

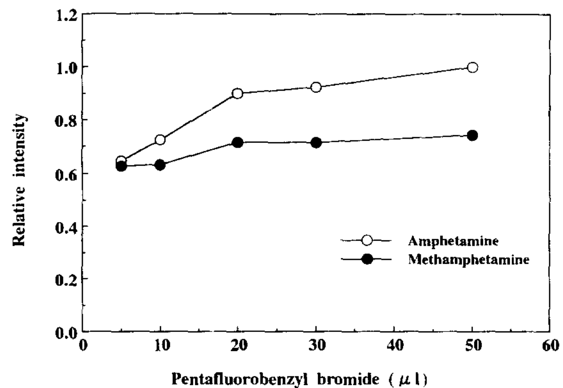

Fig. 7. Effect of added amout of PFBBr on extraction and derivatization of MA and AM. 1 ml Blood spiked with MA (10.31 µg) and AM (9.38 µg) was determined. The longitudinal axis shows relative intensities to standard samples.

Table 1
Precision and accuracy for determination of MA and AM in blood

Compound	Actual[a] (ng ml^{-1})	Measured mean ($n = 5$, ng ml^{-1})	R.S.D.[b] (%)
MA	103	102	2.6
	1146	1136	1.3
AM	97	95	3.0
	938	958	1.6

[a] Blood concentration spiked with MA and AM.
[b] Relative S.D.

3.4. Added amount of triethylamine and PFBBr

As shown in Fig. 6, the production of PFB derivatives was accelerated by the addition of triethylamine compared to no addition. The maximum intensity of PFB derivatives was obtained by adding 20 μl of triethylamine, and further addition gave constant intensity. The amount of triethylamine selected was 30 μl.As shown in Fig. 7, the formation of PFB derivatives largely increased in the range 5–20 μl of PFBBr. PFB derivatives increased slightly with further addition of PFBBr over 20 μl. The amount of PFBBr selected was 30 μl.

3.5. Analysis of practical samples

The calibration curves for MA ($y = 0.3393x + 0.0093$) and AM ($y = 0.4028x + 0.0098$) gave excellent straight lines over the range of 52 ng (with an S/N ratio of 10)–11.46 μg ml^{-1} for MA and 49 ng (with a S/N ratio of 10)–9.38 μg ml^{-1} for AM with correlation coefficients $r = 0.999$. As shown in Table 1, excellent accuracy and precision could be obtained by this method for 1 ml blood spiked with either 103 ng MA and 97 ng AM or 1146 ng MA and 938 ng AM.

This method was applied to two autopsy cases with the possibility of MA toxicity.

The results of two cases are shown in Table 2. Case 1 was an abuser who was murdered and case 2 was an abuser who died of an overdose. Both cases showed a sufficiently high concentration of MA for detection and this method was very useful for determining the MA concentration of toxicity levels. The AM concentration in both cases amounted to less than 3% MA. These results agreed well with the criteria on toxicity levels reported by K. Hara et al. [10].

In conclusion, methamphetamine and amphetamine in blood can be determined with simplicity and rapidity by a simultaneous procedure of extraction and derivatization. It is suggested that this method is useful for forensic and therapeutic toxicology.

Table 2
MA and AM concentrations in blood of autopsy cases

Case No.	MA (μg ml^{-1})	AM (ng ml^{-1})
(1) Murdered, age 44, male	3.83	57
(2) i.v. Overdose, age 19, male	4.70	116

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