TECHNICAL NOTE

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Comparison of Extraction Methods for Methamphetamine and its Metabolites in Tissue

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ABSTRACT: Five extraction methods were examined for analysis of methamphetamine and its major metabolites in tissue samples. The extraction methods studied were an acetone extraction method, an ethanol extraction method, an ammonium sulfate method, dialysis, and a direct solvent extraction. Acetone, ethanol, and dialysis methods showed no interference from endogenous components using thin-layer chromatography and gas chromatography, and gave satisfactory recovery of methamphetamine, amphetamine, and *p*-hydroxymethamphetamine when added to rabbit liver. These methods, however, proved time-consuming. The ammonium sulfate method and direct solvent extraction method were simple and more rapid, but recovery of the polar metabolite was poor.

KEYWORDS: toxicology, methamphetamine, extraction, drug identification

Recently in Japan, the abuse of methamphetamine has markedly increased and has become a serious social problem. The most common method for detection of users is the examination of urine for the drug and its metabolites. In addition, the use of other easily collectable samples such as hair [*1-4*], sweat [*4-6*], saliva [*4*], and nail [*4*] have been reported. Tissue samples taken at autopsy are analyzed using small samples (0.3 to 2 g) and highly sensitive analytical methods such as mass fragmentography and gas chromatography (GC) with a nitrogen-phosphorous detector following direct solvent extraction [*7-12*]. For routine work in forensic science laboratories, thin-layer chromatography (TLC) and GC with flame ionization detection are commonly used with confirmation by gas chromatography/mass spectrometry (GC/MS). Therefore, relatively large amounts of tissues are required for analysis and choice of extraction methods becomes an important issue.

In this paper, five extraction procedures were evaluated in the extraction of methamphetamine and its major metabolites, amphetamine and *p*-hydroxymethamphetamine, from liver, and the recoveries of these compounds and interference of endogenous components in TLC and GC were examined. The extraction procedures employed were (1) solvent extraction after preparation of protein-free aqueous solution using acetone method, (2) ethanol extraction, (3) an ammonium sulfate method, (4) dialysis, and (5) direct solvent extraction.

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Materials and Methods

Materials

Methamphetamine hydrochloride and amphetamine sulfate were purchased from Dainippon Pharmaceutical Co.. Osaka, Japan and Takeda Chemical Industries Ltd.. Osaka, Japan, respectively. *p*-Hydroxymethamphetamine hydrochloride was prepared by the method of Buzas and Dufour [*13*]. All other chemicals were of reagent grade available commercially.

Extraction Procedure

Acetone Method—Ten grams of minced liver tissue was acidified with tartaric acid and homogenized in a blender with twenty-five millilitres of a seventy percent acetone solution. The homogenate was heated on a water bath at 60°C for 30 min and then filtered after cooling. This process was repeated with the residue and the filtrate combined was concentrated to about 5 mL by evaporation *in vacuo*. The concentrated solution was alkalized with aqueous ammonia and again acidified with tartaric acid. After removal of precipitate by filtration, about 40 mL of acetone was added. The mixture was filtered and concentrated to about 2 mL by evaporation *in vacuo*. To residual solution, 40 mL of acetone was added, the mixture was filtered, and the filtrate obtained was evaporated *in vacuo*. The residue was extracted twice with 10 mL of hot 0.1% hydrochloric acid solution and the aqueous extract was filtered. The filtrate combined (protein-free aqueous solution) was adjusted to pH 9 with sodium carbonate and extracted three times with chloroform-isopropanol (3:1). The combined organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo* after adding a few drops of acetic acid to prevent evaporation of amines. The residue obtained (the chloroform-isopropanol extract) was dissolved in 0.2*N* hydrochloric acid washed with ether twice and then extracted with ether three times after alkalization with sodium hydroxide. The ether layer combined was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo* after adding a few drops of acetic acid (Extract I). The aqueous phase that remained was neutralized with hydrochloric acid, realkalized with sodium carbonate, and extracted three times with chloroform-isopropanol (3:1). The combined organic phase was evaporated *in vacuo* (Extract II) after being dried with anhydrous sodium sulfate, filtration, and addition of a few drops of acetic acid.

Ethanol Method—Ten grams of minced liver was acidified with tartaric acid and homogenized in a blender with twenty millilitres of ethanol. The homogenate was refluxed for 1 h, cooled, and filtered. This process was then repeated with the residue and the two filtrates then combined. The ethanol was removed by evaporation. To the viscous residue obtained, 10 mL of hot ethanol was added and the mixture was filtered after cooling. This process was repeated with the residue and the two filtrates combined then evaporated *in vacuo*. The residue obtained was extracted twice with hot 10 mL of 0.1% hydrochloric acid and the extract was filtered. The filtrate was extracted by the same procedure as that for protein-free aqueous solution under acetone method.

Ammonium Sulfate Method—Minced liver, 10 g, was homogenized in a blender with 10 mL of 0.1*N* hydrochloric acid. Solid ammonium sulfate was added to make a saturated solution. The mixture was warmed in a water bath to 60°C until the protein coagulated. The mixture was filtered after cooling, and the residue was re-extracted with 10 mL of hot 0.1% hydrochloric acid and filtered as before. The combined filtrates were left in a refrigerator and the solidified component was removed by filtration. The subsequent procedure was the same as that for protein-free aqueous solution under acetone method.

Dialysis Method—Ten grams of minced liver was homogenized in a blender with twenty millilitres of water. After addition of 2 mL of 1*M* sodium carbonate solution, the mixture was transformed to the Visking tube and dialyzed against 40 mL of chloroform-methanol

(1 : 1) overnight. After addition of chloroform to the outer solution, the organic phase was separated. The remaining aqueous phase of the outer solution was extracted twice with 20 mL of chloroform-isopropanol (3 : 1). All three organic phase were combined, dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* after the addition of a few drops of acetic acid. The residue was treated by the same procedure as that for the chloroform-isopropanol extract under the acetone method.

Direct Solvent Extraction—Minced liver, 10 g, was homogenized in a blender with 25 mL of 0.1 *N* hydrochloric acid, and the homogenate was extracted by the same method as that for protein-free aqueous solution under the acetone method.

Analytical Procedure

TLC was carried out on 0.25-mm-thick silica gel GF254 plates (E. Merck, Darmstadt, G.F.R.); the solvent systems used for development were (1) isopropanol-28% aqueous ammonia (95:5) and (2) acetone-benzene-28% aqueous ammonia (2 : 1 : 0.1). After development, the plates were examined under ultraviolet (UV) light (254 nm) and sprayed with one of the following detection reagents: (a) 1% iodine-methanol solution; (b) 20% sodium carbonate solution and 1% sodium nitroprusside solution as detection reagent, and the plate was exposed to acetaldehyde gas for 1 min; and (c) 0.25% fluorescamine-acetone solution, then trimethylamine-dichloromethane (1 : 10).

A Shimadzu GC-4CM gas chromatograph equipped with a flame ionization detector was used for analysis. A glass column (2-m by 3-mm inside diameter [ID]) was packed with 3% OV-17 on Chromosorb W acid-washed dimethlyldichlorosilane (AW DMCS) (100–120 mesh). The carrier gas was nitrogen (50 mL/min). The column, injector, and detector temperatures were 145, 180, and 180°C, respectively. The extract obtained by each extraction method was dissolved in 200 μL of ethyl acetate and 200 μL of trifluoroacetic anhydride were added. The vessel containing the mixture was sealed tightly and heated at 55°C for 20 min. The solvent was evaporated *in vacuo*, the residue obtained was dissolved in 100 μL of ethyl acetate, and 1 μL was injected into the gas chromatograph. For the determination of methamphetamine and its metabolites, 0.3 μg/μL of fluorene in ethyl acetate was used as an internal standard.

GC/MS was carried out with a Hitachi Model M-80 double-focusing mass spectrometer by the chemical ionization mode using isobutane as the reactant gas. A 1-m by 3-mm ID glass column packed with 3% OV-17 on Chromosorb W AW DMCS (100–120 mesh) was used. The column, injector, and separator temperatures were 125, 165, and 180°C, respectively. The flow rate of the helium carrier gas was 50 mL/min. The ionization voltage was 100 eV and the ionization current was 110 μA.

Results and Discussion

After first chloroform-isopropanol extraction, relatively large amounts of oily residue were obtained by all five extraction procedures, especially the direct solvent extraction method. They showed pronounced tailing of spots on thin-layer chromatograms and a number of extraneous peaks on gas chromatograms. Therefore, the back-extraction and the fractionation into two subgroups (Extracts I and II) were carried out. Methamphetamine and amphetamine were extracted in Extract I and *p*-hydroxymethamphetamine was in Extract II. No interferences were observed on thin-layer chromatograms of all subgroups. All Extracts I gave similar gas chromatograms, which showed very slight extraneous peaks (Fig. 1), and Extracts II gave a few extraneous peaks as shown in Fig. 1, but no peaks had the same retention time as *p*-hydroxymethamphetamine or fluorene (I.S.). Table 1 showed the results of the recovery experiments of methamphetamine and its metabolites when added to rabbit liver. When acetone, ethanol, or dialysis method was applied, the recoveries of three compounds were 70 to 90% except amphetamine (60%) in ethanol method. In the ammonium

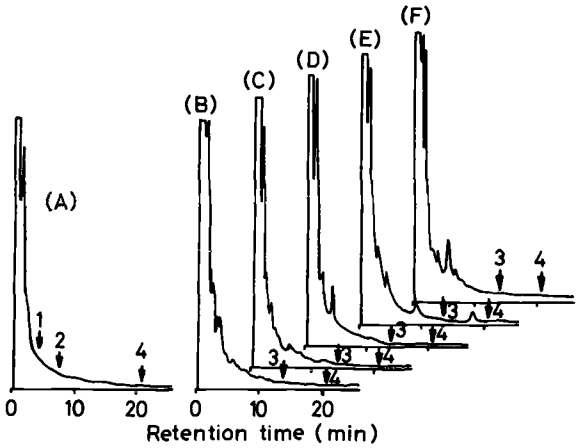

FIG. 1—*Gas chromatograms of trifluoroacetylated derivatives of extracts obtained by different extraction methods.* (a) *Extract I of acetone method (Extracts I of other methods show the similar chromatograms to this);* (b) *Extract II of acetone method;* (c) *Extract II of ethanol method;* (d) *Extract II of ammonium sulfate method;* (e) *Extract II of dialysis method; and* (f) *Extract II of direct solvent extraction method. Arrows show the retention times of trifluoroacetylated derivatives of the authentic compounds.* 1, *Amphetamine;* 2, *methamphetamine;* 3, p-*hydroxymethamphetamine; and* 4, *fluorene.*

TABLE 1—*Recoveries of methamphetamine and its metabolites.*

Extraction Method	Recovery, %[a]		
	Methamphetamine	Amphetamine	*p*-Hydroxymethamphetamine
Acetone method	68.1 ± 1.6	81.9 ± 0.1	72.6 ± 1.7
Ethanol method	72.5 ± 1.9	60.2 ± 5.2	79.9 ± 3.3
Ammonium sulfate method	102.7 ± 5.0	92.8 ± 5.6	47.4 ± 13.0
Dialysis method	69.6 ± 2.2	89.8 ± 3.1	86.7 ± 2.5
Direct solvent extraction method	103.9 ± 2.2	97.7 ± 7.4	48.4 ± 21.5

[a]Fifty micrograms of methamphetamine hydrochloride, five micrograms of amphetamine sulfate, and five micrograms of *p*-hydroxymethamphetamine hydrochloride were added to ten grams of rabbit liver.

sulfate method and the direct solvent extraction method, 93 to 104% of methamphetamine and amphetamine were recovered, but only 50% of *p*-hydroxymethamphetamine was recovered and unsatisfactory reproducibilities (standard deviation [SD] 13 to 21%) were obtained.

These results show the advantages and disadvantages of each extraction procedure. Although the acetone, ethanol, and dialysis methods were very time-consuming, they gave satisfactory recoveries with all compounds and were easily applied to large-scale samples. The ethanol extraction method has been applied to tissues in human case and proved satisfactory (Fig. 2). The dialysis method was very simple but required overnight dialysis. The ammonium sulfate method was more rapid than the acetone, ethanol, and dialysis methods, but recovery varied widely from preparation to preparation. The direct solvent extraction method was rapid and simple, and suited for analysis of small-scale samples, but recovery of the polar metabolite was poor.

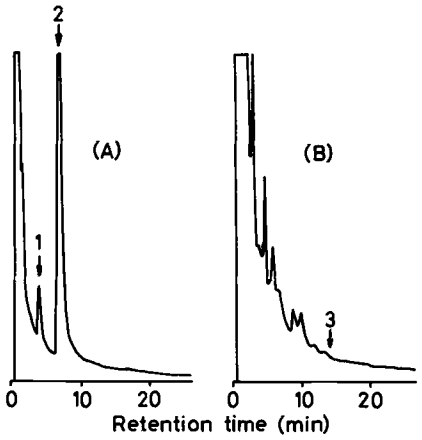

FIG. 2—*Gas chromatograms of trifluoroacetylated derivatives of* (a) *Extracts I and* (b) *II obtained from liver of a methamphetamine suspect abuser. Arrows show the retention times of trifluoroacetylated derivatives of the authentic compounds.* 1, *Amphetamine;* 2, *methamphetamine; and* 3, p-*hydroxymethamphetamine. The two peaks in* (a) *were confirmed as amphetamine and methamphetamine by gas chromatography/mass spectrometry. Although* (b) *gave no peak having the same retention times as* p-*hydroxymethamphetamine,* p-*hydroxymethamphetamine was detected by mass chromatography using a quasimolecular ion peak* (m/z *358) and base ion peak* (m/z *154) of trifluoroacetylated* p-*hydroxymethamphetamine.*

Conclusion

In conclusion, the acetone, ethanol, and dialysis methods are recommended for the analysis of methamphetamine and its metabolites in tissue samples. The direct extraction method is useful for the analysis of methamphetamine and amphetamine, but not for the polar metabolite in small tissue samples.

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