Rapid Extraction of Methamphetamine and Amphetamine in Body Fluids with Bond Elut SCX Cartridges before Capillary Gas Chromatography

[Jpn J Legal Med 47 (2), 129-133, 1993]

TECHNICAL NOTE

Rapid Extraction of Methamphetamine and Amphetamine in Body Fluids with Bond Elut SCX Cartridges before Capillary Gas Chromatography

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(Received Dec.7, 1992; accepted Jan. 8, 1993)

Abstract. A simple and rapid method for extraction of methamphetamine and amphetamine from human whole blood and urine with Bond Elut SCX cation exchanger cartridges is presented. Detection of the stimulants was made by capillary gas chromatography (GC) with flame ionization detection after derivatization with trifluoroactic anhydride. The compound-containing samples, after mixing with 0.05 M potassium phosphate buffer (pH 7.8), were directly applied to the cartridges, washed with 20 ml of 50% methanol in water and eluted with methanol-HCl (240 : 1). The recoveries of both methamphetamine and amphetamine were close to 100%. The background noises for Bond Elut SCX cartridge extraction were much smaller than those for Sep-Pak C_{18} cartridge and diethyl ether extractions.

Key words: Toxicology, Methamphetamine, Amphetamine, Extraction, Bond Elut SCX cartridges

Introduction

Methamphetamine and amphetamine were isolated from biological samples by liquid-liquid extraction with organic solvents in many reports[1)~14)]. However, recently, solid-phase cartridges or columns, such as Sep-Pak C_{18} [15)] (Bond Elut C_{18} [16)]), Extrelut[17)] and Bond Elut Certify™ [18)] have been used for isolation of the stimulants, before their analysis by gas chromatography (GC) or GC/mass spectrometry (MS), because of their simplicity or cleaner backgrounds. In this paper, we present another type of solid-phase cartridge Bond Elut SCX for isolation of the stimulants before capillary GC analysis. The results with the new sorbent have been compared with those with Sep-Pak C_{18} cartridges and liquid-liquid extraction.

Materials and Methods

Chemicals

Methamphetamine-HCl was purchased from Dainippon Pharmaceutical (Osaka); and trifluoroacetic (TFA) anhydride from Pierce (Rockford, IL, USA). Amphetamine sulfate was kindly donated by Dr. Yoshiko Yamamoto, Department of Legal Medicine, Faculty of Medicine, Kyoto University. Other chemicals used were of analytical grade. Bond Elut SCX cartridges were purchased from Varian Sample Preparation Products (Harbor City, CA, USA); Sep-Pak C_{18} cartridges from Waters Associates (Milford, MA, USA); and a fused-silica capillary column (DB-5, 30 m × 0.32 mm i.d., film thickness 0.25 μm) from J & W Scientific (Folsom, CA, USA). Whole blood and urine were obtained from healthy subjects.

GC conditions

GC analyses were carried out on an HP 5890 Series II gas chromatograph with flame ionization detection (Hewlett-Packard Co., Palo Alto, CA, USA). The GC conditions were : column temperature, 90-190°C (10°C/min); injection temperature, 220°C; and helium gas flow, 3 ml/min. The samples were injected in the splitless mode and the splitter was opened after 1 min.

Extraction with Bond Elut SCX cartridges

Bond Elut SCX cartridges were pretreated by passing 10 ml of methanol-HCl (240 : 1), 10 ml of methanol, and 30 ml of distilled water through them. For new cartridges, this procedure was

repeated more than twice to reduce background noises. One-ml urine containing methamphetamine and amphetamine (1 μg each) was mixed with 4 ml of 0.05 M potassium phosphate buffer (pH 7.8); in the case of whole blood, the 1-ml sample was mixed with 9 ml of the buffer for complete hemolysis. Each sample solution was poured into the pretreated cartridge. The cartridge was then washed with 20 ml of 50% (v/v) methanol in water, followed by 3 ml of methanol-HCl (240:1) to elute both compounds from the cartridge. The eluate was evaporated to dryness *in vacuo*.

Extraction with Sep-Pak C_{18} cartridge

Extraction of both stimulants with Sep-Pak C_{18} cartridge was carried out essentially by the method of Suzuki *et al.*[15]. Sep-Pak C_{18} cartridges were pretreated by passing 10 ml of methanol and 20 ml of distilled water. For new cartridges, this procedure was repeated more than twice to reduce background noises. One-ml urine containing methamphetamine and amphetamine (1 μg each) were mixed with 4 ml of 0.05 M glycine buffer (pH 10); in the case of whole blood, the 1-ml sample was mixed with 9 ml of the buffer for complete hemolysis. The sample solution was poured into the pretreated cartridge. The cartridge was then washed with 5 ml of 30% (v/v) methanol in water; finally 10 ml of methanol was passed through the cartridge to elute both compounds from the cartridge. After addition of one drop of acetic acid to the eluate, it was evaporated to dryness *in vacuo*.

Liquid-liquid extraction

Extraction of the stimulants with diethyl ether was carried out as described previously[15]. One-ml whole blood or urine containing stimulants (1 μg each) was mixed with 0.2 ml of 1 M NaOH and 1 ml of distilled water. The mixture was extracted twice with 2 ml of diethyl ether. After addition of one drop of acetic acid, the combined extracts

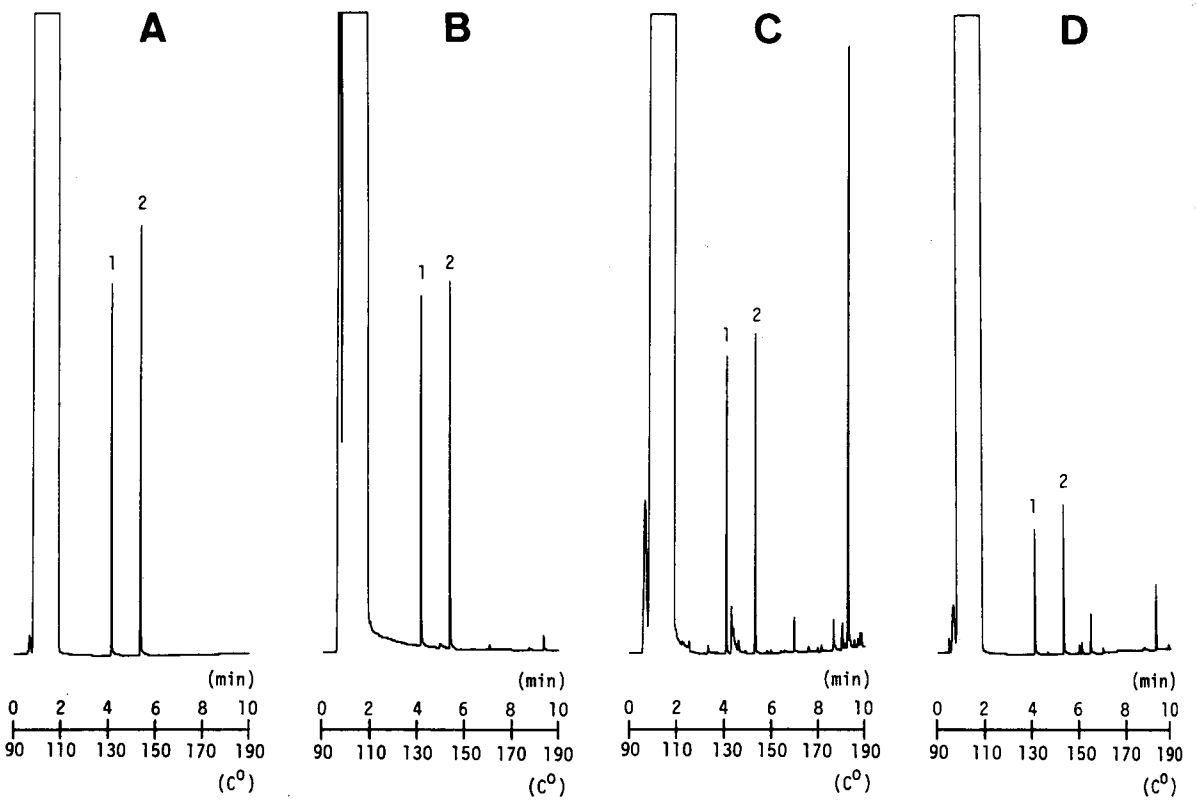

Fig. 1. Capillary GC for TFA derivatives of methamphetamine (peak 2) and amphetamine (peak 1) extracted from human whole blood by various isolation methods. A: the authentic methamphetamine and amphetamine without extraction; B: Bond Elut SCX cartridge; C: Sep-Pak C_{18}; D: diethyl ether. The mixture of methamphetamine and amphetamine (1 μg each) was added to 1 ml of human whole blood.

were evaporated to dryness under a stream of nitrogen.

Derivatization of the stimulants

Both stimulants were derivatized with TFA anhydride. A 200-μl aliquot of TFA anhydride-ethyl acetate (5:1, v/v) was added to each residue and heated at 80°C for 5 min. The solvent was then evaporated to dryness under a stream of nitrogen and the residue was dissolved in 100 μl ethyl acetate; 2 μl of it was subjected to GC analysis.

Results and Discussion

Figures 1 and 2 show gas chromatograms obtained with three extraction methods for methamphetamine and amphetamine that have been spiked to whole blood and urine samples, respectively. The retention times for amphetamine and methamphetamine were 4.2 and 5.4 min, respectively. Both compounds could be satisfactorily separated from each other on the chromatograms. The backgrounds obtained from extraction with Bond Elut SCX cartridges were fairly clean, but those for Sep-Pak C_{18} cartridges and diethyl ether showed many impurity peaks.

Recoveries of methamphetamine and amphetamine for two body fluid samples by use of the three extraction methods are summarized in Table 1. Recoveries were highest with Bond Elut SCX and lowest with ether extraction for both whole blood and urine.

Recently, Gan *et al.*[18] reported that Bond Elut Certify™ bonded silica sorbent cartridge was an effective solid-phase for extraction of methamphetamine and amphetamine. However, the compounds were extracted from only urine samples and the recoveries for methamphetamine and amphetamine were 81 and 66%, respectively; while in the present study, Bond Elut SCX cartridges showed 92.6 and 107% recovery, respectively (Table 1).

From the view points of backgrounds, recovery

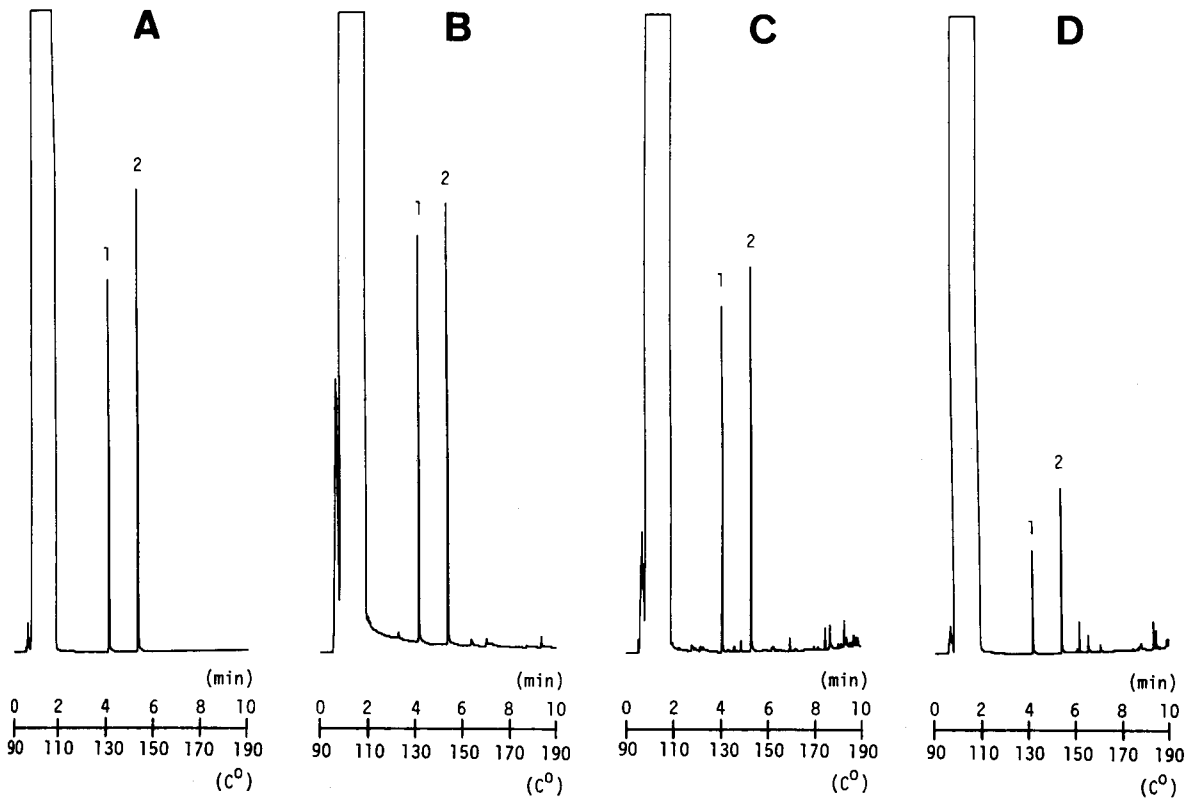

Fig. 2. Capillary GC for TFA derivatives of methamphetamine (peak 2) and amphetamine (peak 1) extracted from human urine by various isolation methods. A: the authentic methamphetamine and amphetamine without extraction; B: Bond Elut SCX cartridge; C: Sep-Pak C_{18} cartridge; D: diethyl ether. The mixture of methamphetamine and amphetamine (1 μg each) was added to 1 ml of human urine.

Table 1. Recoveries of Methamphetamine and Amphetamine from human whole Blood and Urine

Sample	Extraction method	Percent recovery	
		Methamphetamine	Amphetamine
Whole blood	Bond Elut SCX	86.2±1.20	99.7±2.31
	Sep-Pak C_{18}	78.2±5.14	79.9±1.62
	Diethyl ether	36.7±2.64	37.2±3.37
Urine	Bond Elut SCX	92.6±4.30	107 ±3.06
	Sep-Pak C_{18}	80.6±2.69	92.7±2.67
	Diethyl ether	36.3±2.36	31.9±2.60

Each compound (1 μg) was added to 1 ml of human whole blood or urine.
The values are means±SD of 3 experiments.

and simplicity, the present isolation method with Bond Elut SCX seems most recommendable as pretreatment for analyses by GC, GC/MS and also high performance liquid chromatography.

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Bond Elut SCX カートリッジによる体液中メタンフェタミンおよびアンフェタミンの迅速抽出法

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(受付：平成4年12月7日，掲載決定：平成5年1月8日)

摘要 Bond Elut SCX陽イオン交換樹脂カートリッジを用い，ヒト体液中からのメタンフェタミンおよびアンフェタミンの迅速抽出法を設定した．メタンフェタミンおよびアンフェタミンを添加したヒト全血および尿に0.05M リン酸緩衝液 (pH 7.8) を加え攪はんした後カートリッジに注入した．カートリッジを50%メタノール水溶液で洗浄した後，目的物質をメタノール－塩酸 (240：1) 溶液でカートリッジから溶出させ，トリフルオロ無水酢酸で誘導体化し，キャピラリーガスクロマトグラフィー/水素炎イオン化法にて検出した．Bond Elut SCX カートリッジ抽出は，従来用いられていた Sep-Pak C_{18} カートリッジおよびエーテル液－液抽出に比較して，不純ピークの出現が非常に少なく，また，アンフェタミンおよびメタンフェタミンの回収率もほぼ100%と良好で前処理方法としては最適なものと考えられる．