CLEAN-UP PROCESS FOR MASS SPECTRAL STUDY OF AMPHETAMINES IN PUTREFIED BODY MATERIALS

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ABSTRACT: To obtain mass spectra of amphetamines, clean-up before an extractive derivatization through Extrelut column was attempted using putrefied biological materials. By this process, amino acids, organic acids and polyamines were diminished. As a result, reliable mass spectra and concentrations of amphetamines were obtained in an actual case.

KEY WORDS: Amphetamines; Extractive derivatization; Post-mortem materials; GC/MS.

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INTRODUCTION

Mass spectrum is one of most reliable evidences in forensic toxicology. When putrefied biological specimens are used, mass spectral acquisition is usually interfered by many putrefactive substances. In such a case, to obtain intact mass spectra from biological sample, clean-up procedure is needed. Meanwhile, simple and rapid techniques are required for forensic toxicological practice.

We have been developing a simple and rapid sample preparation process by using an Extrelut column in extractive derivatization [1]. However, the prepared samples from post-mortem specimens often contain large amounts of disturbances such as cadaverine, amino acids. Therefore, additive procedure is required for reducing the disturbances.

In this study, one of clean-up processes for obtaining clear mass spectra of amphetamines was investigated.

MATERIALS AND METHODS

Biological materials

For methodological study: blood was provided by a non-drug user. Porcine meat and bovine liver were purchased at a meat store.

For application study: biological materials were collected at autopsy of a cadaver who was killed by use of methamphetamine and left under water in two months.

Reagents

All solvents used in this study were distilled. Extrelut was purchased from E. Merck (Darmstadt, Germany). Heptafluoro-n-butyryl chloride was purchased from Tokyo Chemical Industry (Tokyo, Japan).

Clean-up procedure before extractive derivatization

Biological specimen including methamphetamine-d_5 and 4-hydroxymethamphetamine-d_5 as internal standards was homogenized with 5 times volume of 0.1 M pH 6.0 phosphate buffer, and the supernatant was subjected to sample preparation for amphetamine-analysis. After pH-adjustment at 12.6, the supernatant was applied to an Extrelut column for diethyl ether extraction. The ether extract was back-extracted with 0.01 M HCl. The aqueous solution was washed with diethyl ether, and flashed with nitrogen gas blow. The solution was again adjusted pH to 12.6 for the extractive heptafluoro-n-butyrylation which was described in our previous report [1]. After the sample preparation, the sample was injected into GC/MS.

Conditions of GC/MS

GC/MS analysis was performed by using a Shimadzu QP-5000. XTI-5 used as a separation column was 30 m x 0.25 mm i.d. in a size and 0.25 mm of film thickness. The temperature in column oven was initially kept at 70°C for 1 min after the injection, raised to 290°C by 20°C/min, then held the final temperature until termination. The temperatures of interface and injection port were 260°C and 250°C, respectively. The voltage for ionization was set at 70 eV.

RESULTS AND DISCUSSION

By this clean-up, polyamines (cadaverine, putrescine), amino acids (phenylalanine, tyrosine, etc.) and organic acids (3-phenylpropionic acid, palmitic acid, etc.) detected in the sample prepared by the regular procedure were removed as seen in Figure 1. As primary amines were coextracted with amphetamines, they should be chromatographically separated. Linear standard curves (1 ng to 2000 ng) were obtained of amephetamine and methamphetamine ($r^2 > 0.997$). The reliability of this process was assessed by obtaining the coefficients of variation at 10 ng and 100 ng (n = 5 for each biological material): methamphetamine = less than 3%, amphetamine = less than 8%. As indicated in an actual case, a identifiable mass spectrum of amphetamine was obtained from ca. 0.02 μg of 1 g kidney (Figure 2). In the case shown in this report, concentrations of amphetamine in putrefied biological materials could be only obtained by clean-up samples (Table I).

TABLE I. COMPARISON OF BOTH RESULTS ASSAYED BY RESPECTIVE PROCESSES (AN ACTUAL CASE)

Material*	Clean-up		Regular procedure		
	Amphetamine	Methampheta-mine	Material**	Amphetamine	Methamphe-tamine

Fig. 1. Comparison of chromatographic patterns obtained from 0.02 g of kidney in a poisoning case. By clean-up process, amino acids, organic acids and polyamines were mostly removed (IS-1 = methamphetamine-d5, IS-2 = 4-hydroxymethamphetamine-d5).

Liver	0,26	9,98	Liver	< 0.25	12.31
Kidney	0,14	5,87	Kidney	< 0.10	9.81
Spleen	0,10	4,44	Spleen	< 0.03	4.48
Lung	0,12	5,47	Lung	< 0.08	3.89
Muscle	0,06	3,80	Muscle	< 0.07	3.99

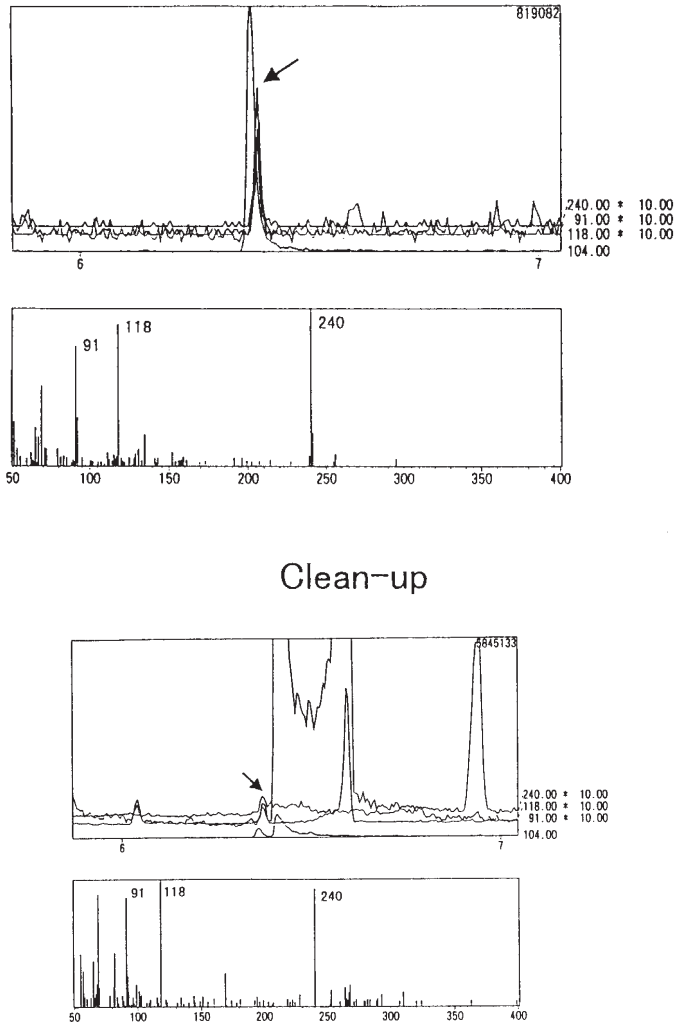

Fig. 2. Comparison of SIM-chromatograms and mass spectra obtained from 0.02 g of kidney in a poisoning case. The efficiency of clean-up can be observed at amphetamine-peak.

Fat	0,01	0,60	Fat	< 0.02	0.63

Unit – μmol/100 g.
* kept at –20°C for 5 months.
** used 2 days after the autopsy (–20°C).

The findings of this study indicated that conventional extraction was effective as clean-up procedure before the extractive derivatization for amphetamine analysis although the recovery of phenolic metabolites decreased.

Reference:

1. Hara K., Kashimura S., Hieda Y., Kageura M, Simple extractive derivatization of methamphetamine and its metabolites in biological materials with Extrelut columns for their GC-MS determination, *Journal of Analytical Toxicology* 1997, vol. 21, pp. 54–58.