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A Rapid and Sensitive Method for Detecting Fenitrothion in Biological Fluids Using the Phosphorus-Sulfur Selective Detector — a fenitrothion intoxication case —

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ABSTRACT

Fenitrothion (sumithion) in biological fluids of a patient, who attempted suicide by ingesting of fenitrothion, was separated and purified by Extrelut® column extraction. A gas chromatograph equipped with a flame photometric detector and a gas chromatograph-mass spectrometer were used for a detection of fenitrothion.

A 41-year-old female, who attempted suicide by ingesting about 30 ml of Sumithion® (40% fenitrothion), started to vomit spontaneously and recurringly, and was transported to a hospital 3 hr after the ingestion. The patient was almost fully conscious and the diameter of her pupils was 3 mm on both sides.

The fenitrothion concentration in the blood sample was 260 ng/g and was less than 6 ng/g in the urine sample both of which were collected 4 hr after ingestion. Aminofenitrothion, 4-nitro-3-methyl phenol, S-methylfenitrothion, phenobarbital and lidocaine were identified in the ethyl ether extract of the urine sample. After ingestion, the serum cholinesterase activity (normal range: 175-440 IU) was 104 at hr, 38 at 1 day, 85 at 2 days, 102 at 3 days and 137 at 4 days.

Fenitrothion [sumithion, 0,0-dimethyl-0-(3-methyl-4-nitro-phenyl) phosphothioate], which is classified as an organophosphorus insecticide, has low toxicity for mammals[12], and is now widely used in the world. Fenitrothion, which is rapidly metabolized into non-toxic compounds in animals[12], has been determined sensitively by using a gas chromatograph (GC) equipped with a flame photometric detector (FPD) operated in the phosphorous mode (526 nm)[1-3,5].

Insecticides in biological materials are usually purified by a method combining liquid-liquid extraction and column chromatography[7]. Fenitrothion in milk[11], fish[18] and tallow[12] are purified either by liquid-liquid extraction, or by the combined method of liquid-liquid extraction and column chromatography. These methods, however, are time-consuming and troublesome.

A simple, rapid and accurate method combining Extrelut® column extraction and gas chromatography and gas chromatography-mass spectrometry has been used for screening and quantifying chemicals in the biological fluids in our laboratory[8-10,13,19-21]. The proposed method combining Extrelut® column extraction and FPD-gas chromatography utilizing both a phosphorus (526 mn) and a sulfur (394 nm) interference filter was developed as a part of our routine drug monitoring methods, and was applied to the analysis of fenitrothion in biologi-

cal fluids of a fenitrothion intoxication case.

Metabolites, the allied substances of fenitrothion and the other drugs in the urine sample were also identified by thin-layer chromatography and gas chromatography-mass spectrometry.

CASE HISTORY

A 41-year-old housewife, who had been treated for depression for approximately 2 years, attempted suicide by ingesting about 30 ml of Sumithion®, containing 40% fenitrothion. Her child found her unable to stand steadily and the patient was taken to a hospital about 3 hr after the ingestion. She was almost fully conscious and the diameter of her pupils was 3 mm on both sides. Fasciculation was not observed. Gastric lavage was carried out in order to prevent further absorption of fenitrothion, although she had vomited up the greater part of the ingested Sumithion® immediately after ingestion. Atropine was given as treatment. She recovered after 5 days of treatment. The serum cholinesterase activity (IU)[15] was 104 at 4 hr, 38 at 1 day, 85 at 2 days, 102 at 3 days and 137 at 4 days after the ingestion.

EXPERIMENTAL

1) Materials:

a) Sample: The patient's blood and urine were collected during the treatment and kept at −20°C until analysis.

b) Control: Drug free urine was obtained from a healthy living man, and blood was collected from a cadaver at autopsy.

c) Chemicals: Fenitrothion; Sumithion® standard (Gas Chro-kogyo Co.). Methidathion; Supracide® (40%, Kumiai Chemical Co.). Extrelut; Extrelut® (Merck Co. Art. 11738). Solvents; reagent grade (Wako Co.).

2) Extrelut column: A 10 cm × 1 cm (i.d.) glass tube packed with 1.0 g of sodium sulfuric anhydride on the bottom layer and 1.2 g of Extrelut on the top layer was used.

3) Gas chromatography: The gas chromatograph (GC) used was a Shimadzu GC-R1A equipped with a FPD utilizing both a 526 nm (for phosphorus) and a 394 nm (for sulfur) interference filter. The column was a 1.0 m × 2.6 mm (i.d.) glass tube packed with 3 w/w% OV-17 on Chromosorb G (60-80 mesh). The temperatures of the injection port, detector and column were set at 270°C, 270°C and 240°C, respectively. Nitrogen with a flow rate of 40 ml/min was used as a carrier gas.

4) Gas chromatography-mass spectrometry: The gas chromatograph-mass spectrometer (GC/MS) used was a Shimadzu GCMS 6020 with a 1 m × 2.6 mm (i.d.) glass tube packed with 3 w/w% OV-17 on Gas Chrom Q (60-80 mesh). Temperatures of the injection port, separator and ion source were set at 260°C, 270°C and 250°C, respectively. The column temperature was programmed from 170°C to 240°C at 8°C/min. The accelerating voltage was 3.5 kV, and the ionization voltages were set at 70 eV for electron impact ionization (EI) and 150 eV for chemical ionization (CI). Helium with a flow rate of 20 ml/min was used as a carrier gas, and isobutane was the reagent gas.

5) Chemical ionization mass fragmentography: The GC/MS and the operating conditions were the same as gas chromatography-mass spectrometry. Monitoring ions used for detecting fenitrothion were m/z 248 and m/z 278.

6) EI mass chromatography: The instrument and the operating conditions column was same as gas chromatography-mass spectrometry.

7) Thin-layer chromatography: The plate used was a Wakogel UA plate (5 cm × 10 cm) including the fluorescent substances. The coloured spots were detected by ultraviolet irradiation (a Topcon PUV-1B) after development by n-hexane:acetone (2:1)[22].

8) Analytical procedure for fenitrothion: The blood sample (0.5 g) was diluted with 0.7 g of water. The diluted blood sample (1.2 g) or the urine sample (1.2 g) and 100 ng of methidathion as an internal standard were poured into the Extrelut column. After letting it stand for 20 min at room temperature, 10 ml of ethyl ether was passed through the column. The eluate was evaporated to dryness under reduced pressure. After the residue was dissolved in 40 μl of ethyl acetate, 2 μl of the solution was analyzed by the GC/MS.

9) Analytical procedure for the metabolites and allied substances of fenitrothion and other drugs: The ethyl ether extracts of the urine sample under neutrality were analyzed by thin-layer chromatography and EI-mass chromatography.

RESULTS

Column extraction: One point two grams of Extrelut used was enough for analyzing the drug in 0.5g of blood or 1.2g of urine. Fenitrothion in sample and methidathion as an internal standard were efficiently extracted by passing 10 ml of ethyl ether through the Extrelut column. The components in the biological fluids and Extrelut, which were extracted simultaneously with the drugs, did not interfere in the detection of these drugs by using a GC-FPD.

Linearity: The calibration curve obtained by plotting the peak height ratios of fenitrothion to the internal standard against the amount of fenitrothion from 0.1 ng to 15 ng was a straight line through the origin as shown in Fig. 1. A correlation coefficient of 0.9997 was obtained.

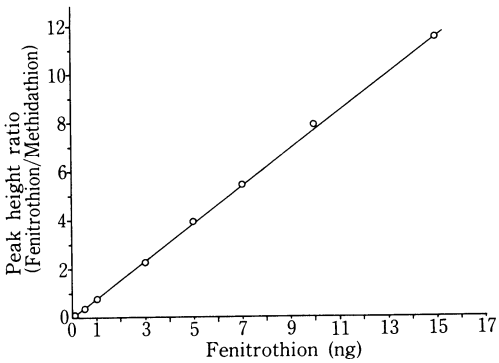

Fig. 1. Calibration curve of fenitrothion

Recovery and precision: Extraction of six replicates of blood or urine each of which contained 60 ng of fenitrothion resulted in a recovery of 80-89% and a coefficient of deviation of 2.5-2.7% as shown in Table 1.

Table 1. Recovery and deviation of fenitrothion

Material	Added (ng)	Found(ng) (X±S)	Recovery (%)	S/X (%)
Blood	60/0.5g	47.9±1.31	79.7	±2.7
Urine	60/1g	53.5±1.33	89.2	±2.5

X: Average. S: Standard deviation. n=6.

Analysis of patient's sample: Fenitrothion in blood sample was detected at 2.0 min by the FPD-gas chromatographic method as shown in peak (1) in Fig. 2, and was identified by the mass spectrum (Fig. 3) and by the CI-mass fragmentographic analysis (Fig. 4). The fenitrothion concentration was 260 ng/g in the blood sample and was less than 6 ng/g in the urine sample both of which were collected 4 hr after ingestion.

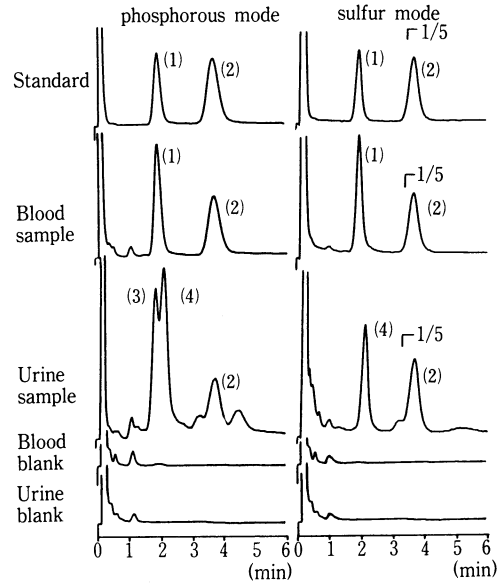

Fig. 2. The FPD-gas chromatograms
Left: a phosphorus type (526 nm)
Right: a sulfur type (394 nm)
(1): fenitrothion, (2): methidation (i.s.)

The detectable limit of fenitrothion by FPD-gas chromatography using a phosphorus mode was almost similar to that by CI-mass fragmentography.

The peak (3) in Fig. 2 was detected by FPD-gas chromatography using the phosphorus mode but not by using the sulfur mode, and not identified by the mass spectrum because the signal was weak. The peak (4) in Fig. 2 was a chemical containing both phosphorus and sulfur according to FPD-gas chromatography. The amount of phosphorus and sulfur, however, was unknown. It was confirmed as aminofenitrothion by the mass spectrum (Fig. 5).

In the blood sample, phenobarbital was also detected by gas chromatograph-mass spectrometry. In the urine sample, 4-nitro-3-methyl phenol, lidocaine, aminofenitrothion, phenobarbital and S-methyl fenitrothion were detected by thin-layer chromatograph, mass spectrum and EI-mass chromatography (Fig. 6). Phenobarbital,

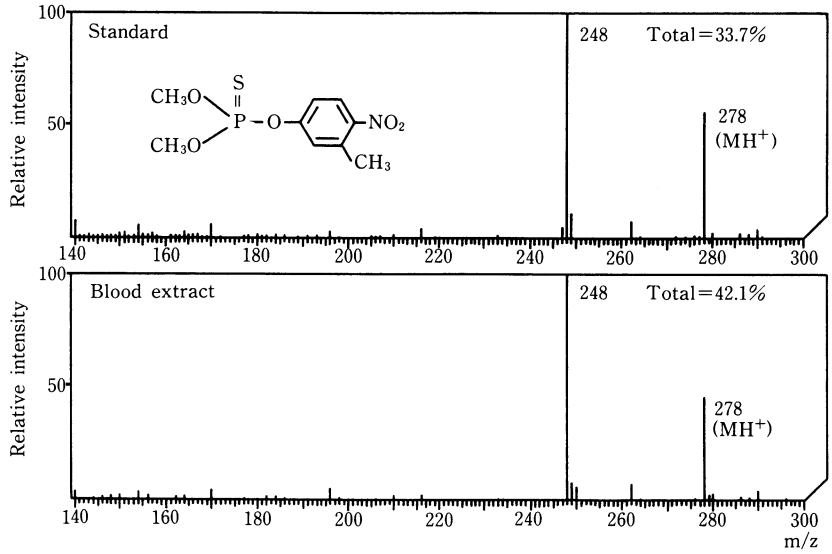

Fig. 3. Chemical ionization mass spectra of fenitrothion

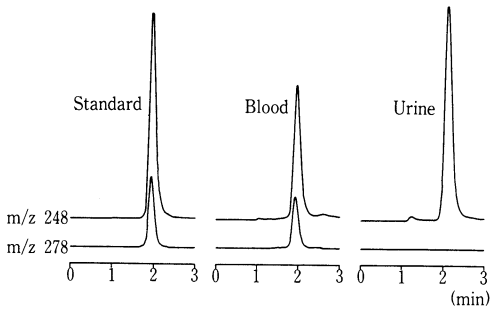

Fig. 4. Chemical ionization mass fragmentograms

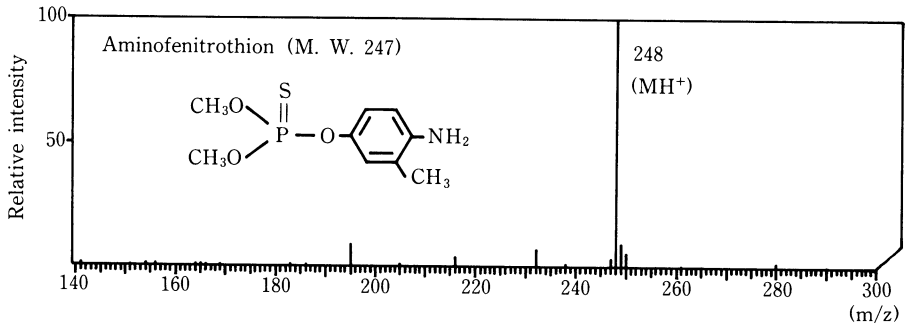

Fig. 5. Chemical ionization mass spectra of aminofenitrothion

aminofenitrothion, 4-nitro-3-methyl phenol and lidocaine, which were extracted from the urine sample with ethyl ether under neutrality and developed with n-hexane: acetone (2:1), had Rf values of 0.48 (reddish yellow), 0.60 (undistinguished colour), 0.61 (green) and 0.63 (pink), respectively.

DISCUSSION

Ecobichon et al[4] and Sakamoto et al[16] reported on human poisoning with fenitrothion. The blood level of fenitrothion, however, is not determined in these reports and was consequently not evaluated. It seems, however, that the blood concentration of fenitrothion of 260 ng/g indicates a low or mild level of fenitrothion poisoning according to the clinical symptom and

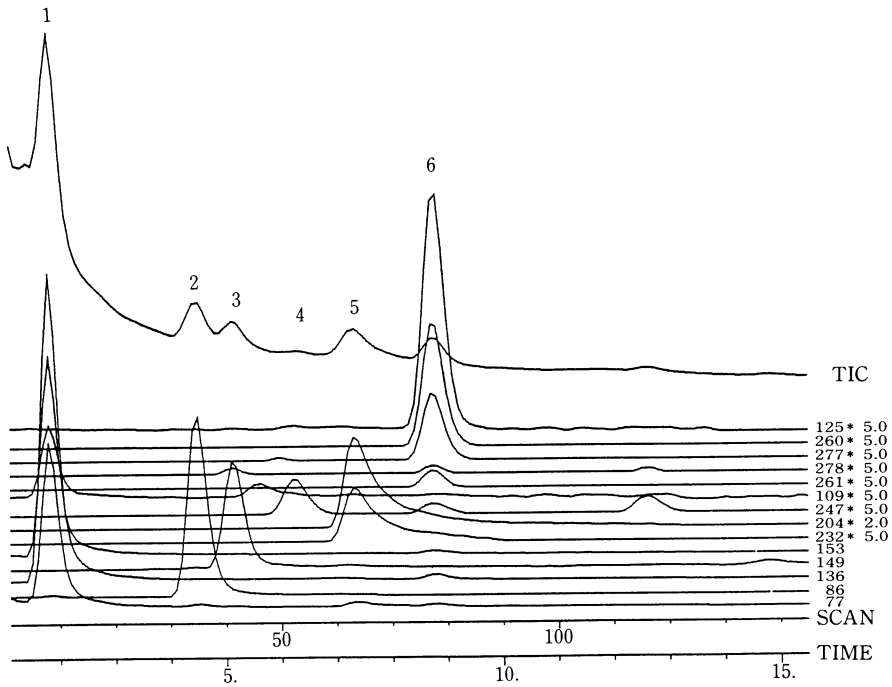

Fig. 6. Electron impact mass chromatogram
1: 4-nitro-3-methyl phenol, 2: lidocaine
3: dibuthyl phthalate, 4: aminofenitrothion
5: phenobarbital, 6: S-methylfenitrothion

the results of the serum cholinesterase activity of this case.

Metabolites and the breakdown products of fenitrothion have been reported as follows; fenitrooxon (FO), aminofenitrothion (AF) and 4-nitro-3-methyl phenol (NMP) in milk[11], FO and NMP in milk[2], desmethylfenitrothion (DMP), NMO and dimethyl phosphorothioic acid in stored wheat[1], S-methylfenitrothion in ground samples[6] and by photodecomposition[14], FO, DMF, NMP and desmethylfenitrooxon in fish[18], AF, NMP, DMF, 3-methyl-4-amino phenol, formylaminofenitrothion and acetylaminofenitrothion in soil[17]. S-methylfenitrothion was identified as a breakdown product after aerial spraying by Greenhalgh and Maeshal[6]. They also report that S-methylfenitrothion is not produced by thermal or on-column re-arrangement on GC, and shows a base ion of m/z 125, a molecular ion (M) of m/z 277 and ion of m/z 260 (M-17). The peak 6 in Fig. 6 was identified S-methylfenitrothion by comparing the mass spectra of S-methylfenitrothion[6]. The compound of the peak (3) in Fig. 2 was not identified by GC/MS, but compound seems to contain same phosphorus but no sulfur, according to the results obtained by FPD-gas chromatography. The Rt value of the compound is similar to that of fenitrooxon reported by Bowman and Beroza[3]. Therefore, the compound seems to be fenitrooxon.

Aminofenitrothion and S-methylfenitrothion, the allied substances of fenitrothion, were found in the urine sample, but not in the blood sample of the fenitrothion intoxication case. The reason why these chemicals were only found in the urine sample and were not detected in the blood sample was unclear.

The phenobarbital found in both the blood sample and the urine sample came from a prescription for insomnia which was caused by depression.

Lidocaine in the urine sample came from a pain depressant jelly used for draining urine.

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