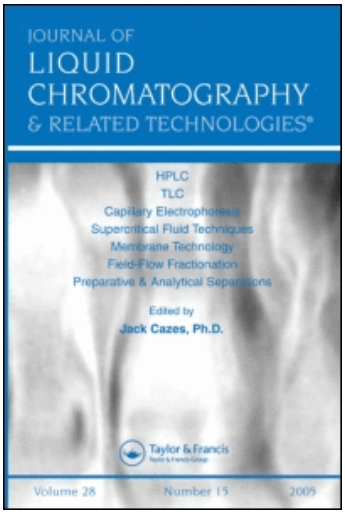

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HPLC QUANTITATION OF FENITROTHION AND 3-METHYL-4-NITROPHENOL IN BIOLOGICAL FLUIDS AFTER PREPACKED CARTRIDGE EXTRACTION AND ITS APPLICATION TO A POISONING CASE

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ABSTRACT

We describe here the simultaneous determination of fenitrothion and its metabolite, 3-methyl-4-nitrophenol (MNP), in biological fluids by HPLC after SepPakC18 cartridge extraction and its application to a human poisoning case. One ml of serum or urine mixed with an equal volume of 0.1 N HCl was applied to the SepPakC18 cartridge, which was preactivated using methanol (5 ml), hexane/isopropanol (9/1 v/v, 10 ml), methanol (5 ml) and distilled water (5 ml). After washing by 0.1N HCl(5 ml), the eluate with 5 ml of hexane/isopropanol (9/1 v/v) was concentrated, and the residue was dissolved in 100 μl of methanol containing methaqualone (internal standard, 30 μg/ml). A 20 μl of the aliquot

was injected into the HPLC. Recovery yields of fenitrothion and MNP from serum and urine ranged from 78.5-93.3%. Linear calibration curves for fenitrothion and MNP were in the range 0.1 - 10 μg/ml. In a human poisoning case at 18.5 hrs after ingestion of Sumithion, concentrations of fenitrothion and MNP in serum were 11.8 and 3.9 μg/ml and those in urine were less than 0.01 μg/ml and 2.8 μg/ml, respectively.

INTRODUCTION

Fenitrothion (Figure 1), an organophosphate insecticide, is widely used in the world. In Japan, an average of 54 deaths per year were registered during the past six years due to fenitrothion poisoning(1).

There has been reported the determination of fenitrothion or its major metabolite, 3-methyl-4-nitrophenol (MNP)(2), using gas chromatography equipped with a flame ionization detector, a flame photometric detector or a mass spectrograph and HPLC(3-6), but reports of their simultaneous determination are few.

We report here the simultaneous determination of fenitrothion and MNP in serum and urine by HPLC after SepPakC18 cartridge extraction and its application to human poisoning cases.

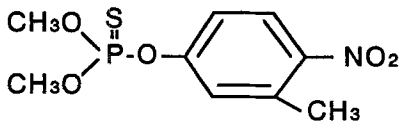

FIGURE 1. Chemical Structure of Fenitrothion.

MATERIALS AND METHODS

Reagents

Fenitrothion (Sumitomo Kagaku), MNP (Aldrich), SepPakC18 cartridge (Waters), methanol (HPLC grade) and the other reagents (Wako Purechemical) were used as purchased. Serum and urine samples were collected from healthy volunteers.

Extraction procedure using a SepPak C18 cartridge

One ml of serum or urine mixed with an equal volume of 0.1 N HCl was applied to the SepPakC18 cartridge, which was preactivated using methanol (5 ml), hexane/isopropanol (9/1 v/v, 10 ml), methanol (5 ml) and distilled water (5 ml). After washing with 0.1N HCl (5 ml), the eluate with 5 ml of hexane/isopropanol (9/1 v/v) was concentrated, and the residue was dissolved in 100 μl of methanol containing methaqualone (internal standard, 30 μg/ml). A 20 μl aliquot was then injected into the HPLC.

Operating conditions of HPLC

The major operating conditions of the HPLC were as follows: Pump; Waters M-600, Column; μBondapakC18 (10 cm x 8 mm i.d.), Detector; Waters-M490, programmed at 310 nm from start to 7 min and at 270 nm after 7 min, Mobile phase; methanol/water (6/4 v/v), Flow rate; 2.0 ml/min.

A poisoning case history

A male (42 year-old-age) ingested Sumithion for suicidal purpose. He was found by his wife 3 hrs after ingestion and gastric lavage subsequently performed. He was then transmitted to our hospital 18.5 hrs after ingestion and given intensive medical treatment such as administration of atropine sulfate (At) and pralidoxime iodide (PAM), direct hemoperfusion (DHP), forced diuresis by dosing of Lasix (furosemide), etc.(7). He left the hospital 65.5 days after ingestion.

RESULTS AND DISCUSSION

Figure 2 shows typical HPLC chromatograms of blank serum (or urine) and of blank serum (or urine) spiked with fenitrothion (1.0 μg/ml) and MNP (1.0 μg/ml). There were no interference peaks for the determination of fenitrothion and MNP in the chromatograms obtained from blank serum (or urine) after SepPackC18 extraction. Each symmetrical peak of MNP, methaqualone (IS) and fenitrothion, which was detected at the retention times of 3.3, 5.2 and 8.4 min, respectively, was clearly separated. No peaks of At, PAM and furosemide were also detected in the chromatogram (data not shown).

Calibration curves were produced using the ratio of peak area of fenitrothion and MNP to that of IS in the HPLC

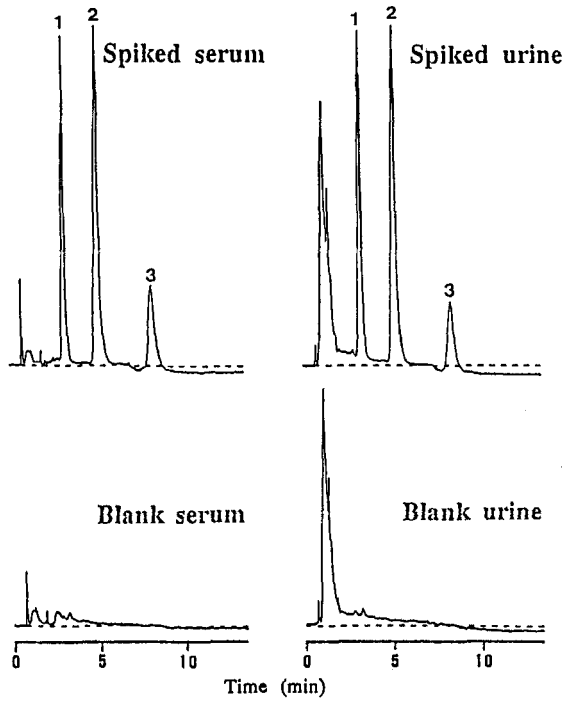

FIGURE 2. HPLC Chromatograms of Serum and Urine Spiked with Fenitrothion (1.0 μg/ml) and 3-Methyl-4-nitrophenol (1.0 μg/ml) and of Blank Serum and Urine after SepPakC18 Extraction. Peaks; 1:3-Methyl-4-nitrophenol 2: Methaqualone (IS) 3: Fenitrothion

chromatograms versus concentration. Figure 3 shows the calibration curves of fenitrothion and MNP. Each calibration curve for fenitrothion and MNP was linear in the range of 0.1 - 10 μg/ml, respectively. The detection limit of fenitrothion was 0.05 μg/ml and that of MNP was 0.01 μg/ml. These detection limits were lower than that obtained by gas chromatography equipped with a

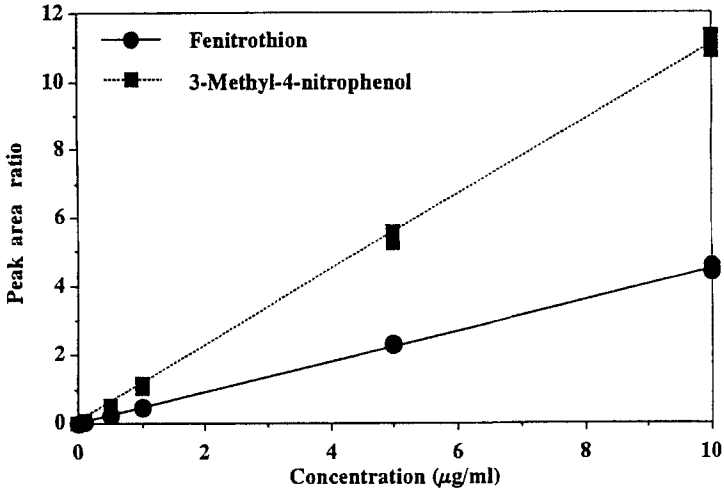

FIGURE 3. Calibration Curves of Fenitrothion and 3-Methyl-4-nitrophenol.

flame photometric detector (4), but sufficiently sensitive for poisoning cases.

Recovery yields of fenitrothion and MNP in serum and urine by SepPakC18 extraction are shown in Table 1. High recovery yields of both fenitrothion and MNP in the range 78.5-93.3% (n=5) were obtained. This extraction procedure is complete within 10 min for the serum and urine samples. The precision of the method was examined using six replicate analyses of the spiked serum and urine at 1.0 μg/ml. The value of the within-day coefficient of variation of the method and the day-to-day were less than 6%. Our quantitation method is relatively rapid and sufficiently sensitive to be useful for poisoning cases due to fenitrothion.

TABLE 1. Recovery Yields of Fenitrothion and 3-Methyl-4-nitrophenol by SepPakC18 Cartridge Extraction.

Sample	Fenitrothion	3-Methyl-4-nitrophenol
Serum (n=5)	**92.1±3.0** (%)	**90.0±2.9** (%)
Urine (n=5)	**78.5±4.3** (%)	**88.2±0.7** (%)

Serum and urine spiked with fenitrothion and 3-methyl-4-nitrophenol (1.0 μg/ml) were used.

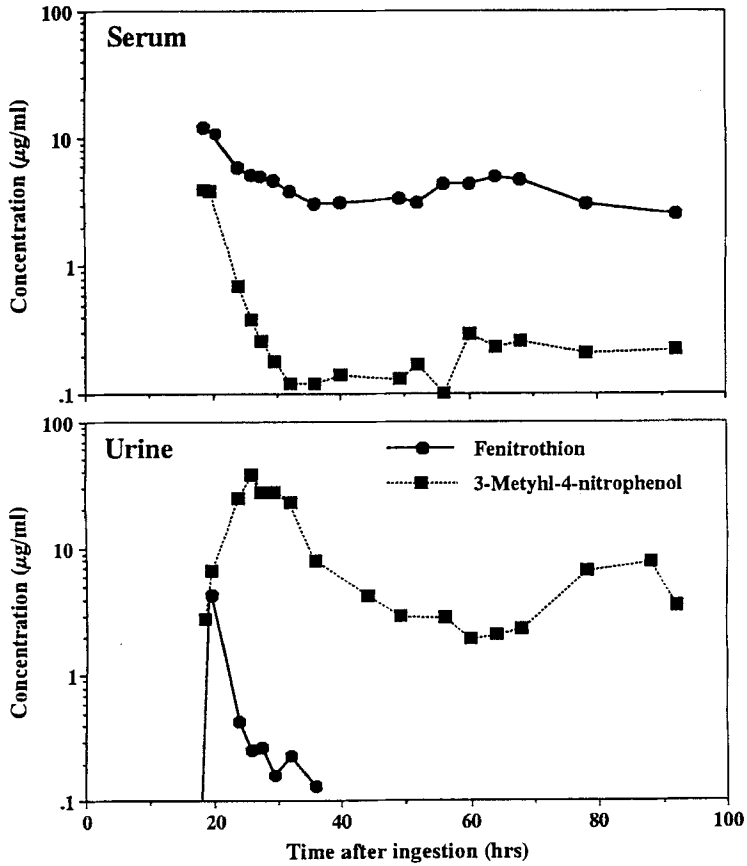

FIGURE 4. Time Course Changes of Fenitrothion and 3-Methyl-4-nitrophenol in Serum and Urine of a Patient Ingested Sumithion.

We applied this method to a poisoning case as shown in Figure 4. Fenitrothion and MNP concentrations in serum at 18.5 hrs after ingestion were 11.8 and 3.9 μg/ml, and those in urine were less than 0.01 μg/ml and 2.8 μg/ml, respectively. In a death case previously reported by Yoshida et al.(8), 16 μg/ml of fenitrothion in the serum was detected. Fenitrothion concentration in the serum detected at the admission was a lethal concentration, but he survived due to our intensive medical treatments. In this case, a decrease in fenitrothion and MNP in serum was rapidly achieved by the emergency treatments such as administration of PAM, forced diuresis and DHP. Fenitrothion in urine also rapidly decreased like that observed in serum, but MNP in urine significantly increased during the first 10 hrs after admission. Activity of acetylcholinesterase inhibited by organophosphate is rapidly reactivated by the administration of PAM(9) the reactivated acetylcholinesterase induces the rapid esterification of organophosphate. Therefore, this significant increase of MNP in urine might be as the results of the rapid esterification of fenitrothion, which produces MNP, by the reactiveted acetylcholinesterase.

REFERENCES

1. National Police Agency, Annual Case Reports of Drugs and Toxic Poisoning in Japan, Vol.30-35, National Police Agency, Tokyo, 1987-1992.

2. H. Aizawa, Metabolic Maps of Pesticides Vol. 1, Academic Press, Tokyo, 1982, p.162.

3. H.Tsukamoto, H.Yoshimura, Y. Nakazawa, M. Uchiyama, Shin Saiban Kagaku, Nanzando, Tokyo, 1972, pp. 209-213.

4. S. Goto, S. Kato, Analytical Methods of Pesticide Residues, Soft-science, Tokyo, 1980, p.104.

5. Y. Kuroiwa, K. Terada, Jpn. J. Toxicol., 1: 67-82(1988)

6. T. Kojima, M. Yashiki, T. Miyazaki, F. Chikasue, M. Ohtani, Forensic Sci. Int., 41: 245-253(1989)

7. M.J. Ellenhorn, D.J.Barceloux, Medical Toxicology: Diagnosis and Treatment of Human Poisoning, Elsevier, NewYork, Amsterdam, London, 1988, pp.1070-1077.

8. M. Yoshida, E. Shimada, S. Yamanaka, Y. Aoyama, Y. Yamamura, S. Owada, Human Toxicol., 6:403-406(1987)

9. P. Taylor, "Anticholinesterase agents", in Goodman and Gilman's The Pharmacological Basis of Therapeutics(8th edn), A.G. Gilman, T.W. Rall, A.S. Nies, P.Taylor, eds, Pergamon Press, NewYork, London, Tokyo, 1990, pp.131-149.

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