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Short Communication

High-performance liquid chromatographic separation of fenthion and its metabolites

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ABSTRACT

The biotransformation of fenthion in animals and plants leads to five major metabolites: fenthion sulphoxide, fenthion sulphone, fenthion oxygen analogue (fenoxon), fenoxon sulphoxide and fenoxon sulphone. According to the FAO, the maximum residue limit for fenthion in different commodities includes the sum of the active ingredient and the above metabolites, expressed as fenthion. In this paper, a reversed-phase high-performance liquid chromatographic method is described which allows a rapid determination of fenthion and its major metabolites. A satisfactory separation was achieved with an RP-18 column and water–acetonitrile (50:50, v/v) as the mobile phase. Under these conditions the detection limits for fenthion and its major metabolites ranged from 0.005 to 0.02 ppm.

INTRODUCTION

Fenthion (**I**, Fig. 1) is a contact and stomach insecticide with a penetrant and persistent action [1]. Owing to its broad spectrum of activity, fenthion is used against fruit flies, leaf hoppers, leaf miners, leaf-eating larvae, stem borers, bugs and other insect pests in fruit, vines, olives, vegetables, rice, beet, etc. It is also employed to control insect pests in public health situations, animal houses and animal ectoparasites [2]. Fenthion is especially useful for the control of fruit flies in many crops, where its ability to penetrate plant tissues allows the destruction of larvae within the fruit.

Five metabolites of fenthion (Fig. 1) have been isolated in animals, being characterized as fenthion sulphoxide (**II**), fenthion sulphone (**III**), fenthion oxygen analogue (fenoxon, **IV**), fenoxon sulphoxide (**V**) and fenoxon sulphone (**VI**) [3].

The biotransformation of fenthion in plants is basically similar to that occurring in animals, the major metabolite being **II**, while **III**–**VI** are present at much lower levels [4]. With regard to milk, **V** is the major metabolite (*ca.* 97%) [5].

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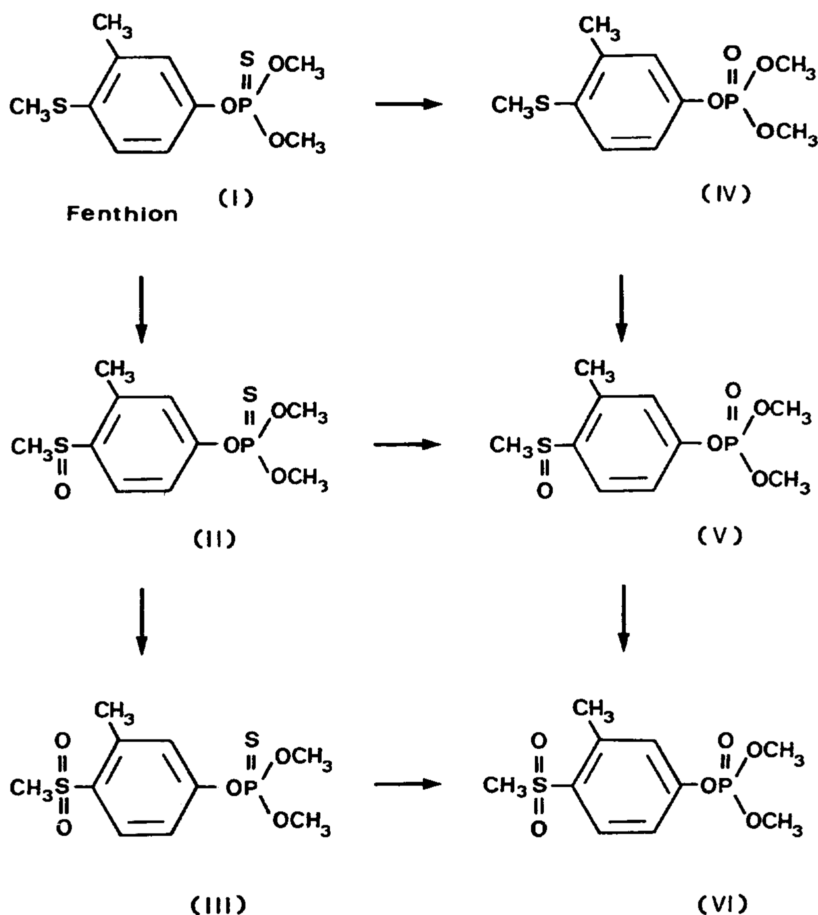

Fig. 1. Fenthion (**I**) and its major metabolites (**II–VI**).

According to the FAO, the maximum residue limit for fenthion in different commodities includes the sum of the active ingredient and its metabolites **II–VI**, expressed as fenthion [6]. This limit is based on an analytical method consisting in the oxidation of **I** and metabolite **II** to **III** and of metabolites **IV** and **V** to **VI**. Compounds **III** (fenthion sulphone) and **VI** (fenoxon sulphone) were then transformed into respective phenols, derivatized and determined by gas–liquid chromatography [7]. This procedure, however, was time consuming and complex. Bowman and Beroza [8] proposed another method, based on the liquid chromatographic separation of **I–VI** into three different fractions, which were analysed separately by gas chromatography with flame photometric detection. Subsequently, they accomplished the simultaneous gas chromatographic separation of the six compounds [9].

In previous work on the metabolic pathway of fenthion, the active ingredient and its metabolites were always determined by gas chromatography. In this paper, a reversed-phase high-performance liquid chromatographic method is described, which allows the rapid determination of fenthion and its major metabolites.

EXPERIMENTAL

Apparatus

A Model 5020 liquid chromatograph (Varian, Palo Alto, CA, U.S.A.) was employed, equipped with a UV-100 variable-wavelength UV–VIS detector and a Rheodyne injector (50-μl loop), connected to a Model 3390 A reporting integrator (Hewlett-Packard, Avondale, PA, U.S.A.).

Chromatography

Hibar (Merck, Darmstadt, Germany) RP-8 and RP-2 (10 μm) columns (250 mm × 4.0 mm I.D.) were used; the mobile phase was water–acetonitrile in various ratios at a flow-rate of 1.0 ml/min. Based on UV spectra, a wavelength of 200 nm was chosen for the simultaneous determination of **I**–**VI**.

Chemicals and materials

Acetonitrile was of HPLC grade (Carlo Erba, Milan, Italy) and acetone, benzene, chloroform, glacial acetic acid, methanol and *n*-hexane of analytical-reagent grade (Carlo Erba). Water was doubly distilled and filtered through a Milli-Q apparatus (Millipore, Molsheim, France) before use. Anhydrous sodium sulphate, hydrogen peroxide, potassium permanganate, selenium dioxide, sodium carbonate, sodium chloride and sodium hydrogencarbonate were of analytical-reagent grade (Carlo Erba).

Fenthion (O,O-dimethyl-O-4-methylthio-*m*-tolyl phosphorothioate, **I**) was an analytical standard purchased from Erhenstorfer (Augsburg, Germany); fenoxon (O,O-dimethyl-O-4-methylthio-*m*-tolyl phosphate, **IV**) was an analytical standard kindly donated by Bayer (Leverkusen, Germany). Fenthion sulphoxide (O,O-dimethyl-O-4-methylsulphinyl-*m*-tolyl phosphorothioate, **II**) and fenthion sulphone (O,O-dimethyl-O-4-methylsulphonyl-*m*-tolyl phosphorothioate, **III**) were synthesized by oxidation of technical-grade fenthion supplied by Bayer, as follows.

With regard to **II**, a solution of equimolar amounts of hydrogen peroxide and selenium dioxide in methanol was added, dropwise and on a magnetic stirrer at room temperature, to a solution of fenthion in methanol. The reaction mixture was then agitated for 15 min, diluted with saturated sodium chloride solution and extracted with chloroform. The chloroform layers were dehydrated with anhydrous sodium sulphate and evaporated, under reduced pressure, to give the crude fenthion sulphoxide as a yellow oil. In order to obtain **III**, an aqueous 0.15 *M* solution of potassium permanganate was added, with stirring at room temperature, to a solution of fenthion in acetic acid. The reaction mixture was agitated for 12 h, worked up by the usual procedure, diluted with water and extracted with chloroform. The organic layers were then neutralized with aqueous sodium hydrogencarbonate, washed with water and dehydrated with anhydrous sodium sulphate. Removal of the solvent under vacuum left a residue of crude fenthion sulphone as a white solid.

Fenoxon sulphoxide (**V**) and sulphone (**VI**) were obtained by the same procedures as used for the respective fenthion analogues, starting from O,O-dimethyl-O-4-methylthio-*m*-tolyl phosphate, which was prepared by reaction of dimethyl phosphorochloride with 3-methyl 4-methylthiophenol in acetone in presence of sodium carbonate, according to the literature [10].

TABLE I

RETENTION TIMES OF FENTHION (I) AND ITS METABOLITES (II–VI) WITH DIFFERENT COLUMNS AND MOBILE PHASE COMPOSITIONS

Column	Water–acetonitrile (v/v)[a]	Retention time (min)					
		V	VI	II	IV	III	I
RP-8	40:60	2.52	2.80	3.63	4.08	4.18	7.50
	50:50	2.73	3.25	4.80	5.76	6.35	14.06
	60:40	3.20	4.26	8.02	10.45	12.60	42.81
	70:30	4.50	6.84	19.09	27.53	34.27	
RP-2	50:50	3.42	3.47	3.87	3.89	3.96	5.60
	60:40	3.76	3.87	5.12	5.36	5.89	12.27
	70:30	4.46	4.89	8.49	9.45	11.92	35.44
	80:20	6.30	7.87	23.29			

[a] The flow-rate was always 1 ml/min.

All the products were purified by chromatography on a silica gel column eluted with suitable benzene–acetone and *n*-hexane–acetone mixtures and their identities were confirmed by means of elemental analysis and IR and ^1H NMR spectrometry. Stock standard solutions (*ca.* 1000 ppm each) were prepared in acetonitrile; working standard solutions were obtained by dilution with the mobile phase.

RESULTS AND DISCUSSION

The separation of fenthion and its metabolites was initially tried with normal-phase columns (NH_2 or CN) and different mobile phases (acetonitrile, acetonitrile–water, methylene chloride and isooctane–ethanol), but in no case was it possible to

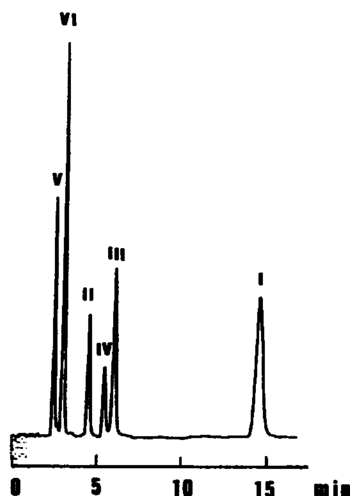

Fig. 2. Chromatography of fenthion (I) and its metabolites (II–VI) on an RP-8 column. Mobile phase, water–acetonitrile (50:50, v/v); flow-rate, 1 ml/min; detection, UV at 200 nm.

separate more than three compounds in one run. Reversed-phase columns were then used (RP-8 and RP-2) with water–acetonitrile mixtures as eluent. The results in Table I show that using the RP-8 column, the separation of **I–VI** can be achieved when the water content in the mobile phase is greater than 40%. On increasing this percentage, the separation also increases, but the peak sharpness decreases, especially for compounds that are more retained on the column, *e.g.*, fenthion. The optimum eluent for a satisfactory separation and a good peak sharpness was water–acetonitrile (50:50, v/v) (Fig. 2).

In comparison with the RP-8 column, the RP-2 column showed higher retention times for **V**, **VI** and **II** and lower retention times for **IV**, **III** and **I**, allowed the separation of **I–VI** with a higher water content in the mobile phase (70%), but led to a poorer peak sharpness (owing to the lower percentage of acetonitrile).

Calibration graphs for each compound were constructed by plotting concentration *vs.* peak height. Good linearities were achieved in the range 0–2 ppm with correlation coefficients between 0.9976 and 0.9993. Under the optimum conditions, the detection limits ranged from 0.005 to 0.02 ppm. UV spectra of fenthion and its metabolites showed absorbance peaks at different wavelengths: 252 nm for **I** and **IV**, 245 nm for **II** and **V** and 225 nm for **III** and **VI**; however, the absorbance of **I–VI** at 200 nm was considerably higher (2–4 times). This allows one to choose the optimum analytical wavelength depending on the concentration of residues in the sample to be analysed and the presence of interfering compounds.

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