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Journal of Chromatography B, 695 (1997) 227–236

JOURNAL OF CHROMATOGRAPHY B

Analytical method for the determination of urinary 3-phenoxybenzoic acid in subjects occupationally exposed to pyrethroid insecticides

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Received 18 November 1996; received in revised form 4 March 1997; accepted 14 March 1997

Abstract

The determination of urinary 3-phenoxybenzoic acid enables exposure to pyrethroid insecticides to be evaluated. A method for the quantitative determination of this metabolite in urine is described. The compound and the internal standard (2-phenoxybenzoic acid) are derivatized with pentafluorobenzylbromide and transformed into pentafluorobenzyl esters, which are determined by gas chromatography with an intermediate polarity capillary column and an electron-capture detector. Before GC analysis, the urinary extracts are purified on LC-Si SPE columns. The proposed method has a detection limit of 0.5 μg/l and a mean recovery of 91.3%. The coefficient of variation of the analytical procedure, evaluated at a concentration of 24.96 μg/l, was 9.58%. Storage of the urine samples for 3 months at −18°C did not lead to significant changes in the concentration of analyte. The method was tested analysing the urine of a farm worker with symptoms of pyrethroid poisoning, occupationally exposed to esfenvalerate. © 1997 Elsevier Science B.V.

Keywords: 3-Phenoxybenzoic acid

1. Introduction

Synthetic pyrethroids are a large class of pesticides introduced in 1980. By 1982 these compounds had captured about 30% of the world insecticide market [1]. Besides widespread use on crops (cotton, maize, soy beans, tobacco, coffee, rapeseed, wheat, barley, alfalfa, vegetables and fruit), synthetic pyrethroids are also used in households, greenhouses and in collars for domestic animals. These compounds are also used to control fleas [2] and scabies [3].

The synthetic pyrethroids considered in this study have an ester bond with 3-phenoxybenzyl alcohol in which the benzyl hydrogen may or may not be substituted with a cyano group. The main members of this group are listed in Table 1.

Skin paraesthesia has been observed in farmers exposed to pyrethroid esters containing a cyano group in position with respect to the 3-phenoxybenzyl alcohol group (deltamethrin, cypermethrin and fenvalerate) [4,5]. A recent study [6] reported 573 cases of acute poisoning during occupational use of deltamethrin and fenvalerate, the symptoms including dizziness, burning of the skin, itching and pins-and-needles sensation. Symptoms disappeared 24 h after exposure. There is no evidence that these

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PII S0378-4347(97)00190-4

Table 1
Main pyrethroid insecticides having an ester bond with 3-phenoxybenzyl alcohol

Common name	Chemical name (from Chemical Abstract)
Acrinathrin	Cyano(3-phenoxyphenyl)methyl 2,2 dimethyl-3-[3 oxo-3-[2,2,2-trifluoro-1-(trifluoromethyl)ethoxyl]-1-propenyl] cyclopropanecarboxylate
Cycloprothrin	Cyano(3-phenoxyphenyl)methyl 2,2 dichloro-1-(4-ethoxyphenyl)cyclopropanecarboxylate
Cyhalothrin	[1α,3α(Z)]-(±)-Cyano(3-phenoxyphenyl)methyl 3-[2 chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate
Cypermethrin	Cyano(3-phenoxyphenyl)methyl 3-(2,2 dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
Cyphenothrin	Cyano(3-phenoxyphenyl)methyl 2,2 dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate
Delthametrin	[1*R*-[1α(*S**),3α]]-Cyano(3-phenoxyphenyl)methyl 3-(2,2dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate
Fenpropathrin	Cyano(3-phenoxyphenyl)methyl 2,2,3,3-tetramethylcyclopropanecarboxylate
Fenvalerate	Cyano(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzeneacetate
Flucythrinate	Cyano(3-phenoxyphenyl)methyl 4-(difluoromethoxy)-α-(1-methylethyl)benzeneacetate
Fluvalinate	Cyano(3-phenoxyphenyl)methyl N-[2-chloro-4-(trifluoromethyl)phenyl]-valinate
Permethrin	(3-Phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
Phenothrin	(3-Phenoxyphenyl)methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)-cyclopropanecarboxylate
Tralomethrin	(3-Phenoxyphenyl)methyl 2,2-dimethyl-3-(1,2,2,2-tetrabromoethyl)-cyclopropanecarboxylate

substances have chronic toxicity in man or animals [7–10].

Because of the ester bond with 3-phenoxybenzyl alcohol, the pyrethroid insecticides in Table 1 are subject to hydrolysis in the human body to form various metabolites including 3-phenoxybenzoic acid which is excreted in the urine as taurine conjugate, glycine conjugate, glutamic acid conjugate and glucuronide [7–10]. Non specific carboxyesterases associated with the microsomal fraction of tissues are involved in the detoxification of these compounds in mammals [11]. A simple metabolic diagram, which by structural analogy also applies to deltamethrin, permethrin, cypermethrin, phenothrin, cyhalothrin and cyphenothrin, is shown in Fig. 1.

Studies on the absorption, distribution, metabolism

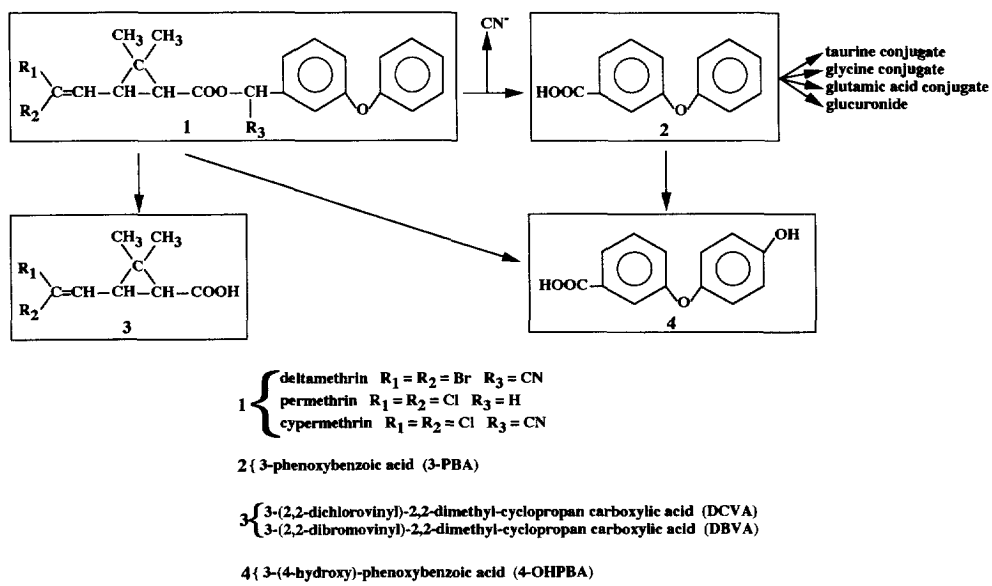

Fig. 1. Metabolic diagram for pyrethroid insecticides with ester bonded 3-phenoxybenzyl alcohol.

and excretion of synthetic pyrethroids have been carried out during therapeutic use of these compounds [12,13]. Ten scabies patients (five men and five women) had about 25 g (range 21–32 g) of a 5% permethrin cream applied to the skin of the whole body, with the exception of the head and neck. Dermal absorption of permethrin was calculated from the quantity of conjugated and nonconjugated *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropan carboxylic acid (DCVA) metabolites of permethrin determined in urine. In samples of urine collected by seven patients one and two days after application of the permethrin cream, 414 and 439 μg mean total DCVA were found, respectively. The mean total DCVA in the urine of three patients who collected their urine in the same container for two days was 1435 μg. The urinary concentration of *trans*-DCVA varied during the first 48 h from 0.11 to 1.07 μg/ml and that of the *cis*-isomer from 0.02 to 0.21 μg/ml. DCVA was still detectable in the urine of three patients after a week and in the urine of one patient, reported to be an alcoholic, after two weeks. The absorption of permethrin over the first 48 h after application was estimated from urinary DCVA excretion levels to be 6 mg (range, 3–11 mg), i.e., 0.5% of the dose applied [12].

Experimental studies into the biotransformation of pyrethroid insecticides in volunteers have been limited but have revealed urinary metabolites derived from esterase-oxidative breakdown of these molecules. *cis* and *trans* forms of 3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropan carboxylic acid (DCVA), 3-phenoxybenzoic acid (3-PBA) and 3-(4-hydroxy)-phenoxybenzoic acid (4-OHPBA) were found in urine after administration of cypermethrin to volunteers [14,15]. After a single oral dose of 0.25–0.50–0.75 mg of cypermethrin, a mean of 43% of the dose was excreted in the urine as DCVA in the first 24 h. A further 49% was eliminated by the volunteers in the next 24 h. No increase in excretion of the metabolite was found when oral administration was carried out in repeated doses. About 0.1% of a dermal dose of 25 mg of cypermethrin was excreted as urinary DCVA within 72 h [14]. In a later study [15] after oral administration of 3.3 mg of cypermethrin, the total quantity of DCVA excreted in the urine collected over 5 days was quantitatively equivalent to that of total phenoxy derivatives (3-PBA+4-OHPBA). The peak of excretion occurred 8–24 h after the dose. On the basis of the DCVA excreted, the quantity of cypermethrin absorbed was 27–57% (mean 36%) of the dose. When 31 mg of cypermethrin was administered dermally, the urinary excretion peak of the metabolites occurred 12–36 h afterwards. In this case, DCVA was quantitatively a quarter of the total phenoxy derivatives (3-PBA+4-OHPBA) in urine. On the basis of the recovery of phenoxybenzoic metabolites, the authors estimated that 0.85–1.80% (mean 1.20%) of the dose administered was absorbed. The results of this study show that for the purposes of biological monitoring of subjects occupationally exposed to this class of compounds, urinary excretion of DCVA could underestimate absorption if the latter is prevalently cutaneous. The total quantity of 3-phenoxybenzoic acid derivatives would provide a better measure of absorption by all possible routes [15].

Previous methods of biological monitoring in subjects occupationally exposed to pyrethroid insecticides have mostly been based on the assay of urinary excretion of non metabolized compounds [16–19] or cyclopropane metabolites [17,19]. Very few studies have been concerned with the assay of phenoxybenzoic derivatives. One of these [20], evaluated absorption of cypermethrin by assaying urinary DCVA, 3-PBA and 4-OHPBA. *cis*-DCVA was undetectable in all urine samples, *trans*-DCVA was found in a limited number of samples (2 or 3 urine samples from two of the three subjects monitored) in quantities ranging from 1.1 to 4.6 μg/l. The other two metabolites were detectable in many of the urine samples of all subjects in concentrations ranging from 1 to 5.7 μg/l for 3-PBA and from 1 to 6 μg/l for 4-OHPBA.

Determination of the compounds exactly as they are excreted in urine makes biological monitoring of a single insecticide possible but has the disadvantage that the analytical results may be falsified by contamination of the sample by hands or clothes soiled with pesticide. The assay of cyclopropane derivatives (DCVA and DBVA) is not very indicative when exposure is only cutaneous and only permits biological monitoring of pyrethroid insecticides with dichlorocyclopropane and dibromocyclopropane groups. Assay of 3-PBA and 4-OHPBA makes it possible to monitor workers exposed to the pyre-

throid insecticides reported in Table 1. The two compounds are therefore suitable indicators of exposure in most situations.

Extremely few analytical methods for urinary metabolites of pyrethroid insecticides have been described [15,20]. Those reported involve derivatization with pentafluorobenzyl bromide [20] or anhydrous pentafluoropropionate in the presence of 1H,1H-pentafluoropropanol [15], followed by GC analysis with capillary column and mass spectrometric detection.

In the present study, we report an analytical method based on GLC with electron-capture detection for the determination of urinary residues of 3-phenoxybenzoic acid. The method, already tested on a farmer exposed to esfenvalerate (the *SS* stereoisomer of fenvalerate) and showing typical symptoms of pyrethroid poisoning (unpublished data), has good precision, excellent recovery and the same detection limit (0.5 μg/l) as one of the non-routine methods mentioned above [15].

2. Experimental

2.1. Reagents and standards

The solvents used to prepare solutions of known titre, for extraction of samples and for purification on silica SPE columns were of the Merck LiChrosolv type (Bracco, Milan, Italy). Pentafluorobenzyl bromide (PFBBr, 99% purity) used for derivatization of the samples was from Aldrich (Milan, Italy). Anhydrous sodium sulphate (>97% purity) used to dehydrate the extracts was from Carlo Erba (Milan, Italy). Silica solid-phase extraction (SPE) columns (3 ml column reservoir, 500 mg sorbent) were from Supelchem (Milan, Italy). They were eluted at 1 ml/min on an SPE vacuum manifold from the same company. Ultrapure water was obtained by the Millipore Milli Q system (Milan, Italy). Standard 3-phenoxybenzoic acid (99.5% purity) was obtained from Lab Service Analytica (Bologna, Italy). The internal standard, 2-phenoxybenzoic acid (2-PBA, >98% purity), was from Fluka (Milan, Italy).

Standard 3-PBA solution at a concentration of 0.5 mg/ml was prepared by dissolving appropriate quantities of standard in methanol. The solutions (working solutions) used to construct the calibration curves in urine were prepared daily, diluting the standard solution with water. Standard 2-PBA solution at a concentration of 1 mg/ml was prepared by dissolving appropriate quantities of standard in methanol. Working solution (1 μg/ml) was prepared daily, diluting the standard solution with water.

2.2. Apparatus

The GC was a Hewlett-Packard 5890A with Model 400 electron-capture detector (ECD) by the same company (Hewlett-Packard Italiana, Milan, Italy) and a Perkin-Elmer 1020 integrator (Milan, Italy). The chromatographic capillary column was Supelco SPB-608, 30 m×0.25 mm, film thickness 0.4 μm (Supelchem). Detector temperature was 350°C. Ultrapure helium carrier was used at a flow-rate of about 1.5 ml/min and argon–methane (95:5) (ultrapure compressed) was used as make-up gas at a flow-rate of 65 ml/min. Injection (1 μl) was performed by the splitless technique. Injector temperature was 300°C. Evaporation of urine extracts was performed in vacuo with a Büchi 011 rotating evaporator and a Büchi 461 water bath (Büchi Laboratoriums-Technik, Flawil, Switzerland). Vacuum was maintained with a Jet 1-Automatic Vacuum System recycled water pump (Genser Scientific Instruments, Rothenbourg, Germany).

2.3. Analytical procedure

A 5-ml volume of urine, spiked with 100 μl of an aqueous solution (1.0 μg/ml) of 2-PBA (internal standard, I.S.) and 1 ml of concentrated H_2SO_4, was kept in a water bath at 100°C for 2 h. When the sample had cooled it was extracted twice with 5 ml dichloromethane in a mechanical shaker. The pooled extracts were dehydrated with anhydrous sodium sulphate and evaporated to dryness in a rotating vacuum evaporated at 30°C. The residue was derivatized by adding 200 μl of an acetone solution of PFBBr (dilution 1:100 of pure PFBBr), 15 μl K_2CO_3 solution (60% w/v in water) and 4 ml acetone. The mixture was briefly shaken in a vortex apparatus and left to react overnight at room temperature. The mixture was then diluted with 5 ml water and extracted with two 2-ml fractions of hexane. The

pooled extracts were dehydrated with anhydrous Na_2SO_4 and evaporated to dryness in a rotary vacuum evaporator at 30°C.

The residue was made up with 1 ml hexane and purified on silica SPE columns previously conditioned with 2 ml toluene and 4 ml hexane. The column was washed with 5 ml hexane and 2 ml hexane–toluene (70:30). Elution of the PFB esters of 2-PBA and 3-PBA was then carried out with 8 ml of hexane–toluene (60:40). The eluate was evaporated to dryness under a gentle stream of nitrogen, made up with 0.5 ml hexane and injected into the GC. The four-step temperature programme was 50°C, 170°C, 215°C and 250°C for 0.9, 0.1, 45 and 20 min, respectively; the rate of temperature increase was 35°C/min from 50°C to 170°C and from 215°C to 250°C and 5°C/min from 170°C to 215°C.

2.4. Calibration curves

3-PBA was determined by calibration curves constructed with solutions prepared by adding appropriate quantities of the working solutions to 5-ml aliquots of urine from a subject not occupationally exposed to pyrethroid insecticides. A sample of the same urine not spiked with standard 3-PBA (blank) was also prepared. The additions were in the range 0–200 μg/l of urine.

2.5. Analytical and GC reproducibility and recovery

A 150-ml pool of urine was spiked with 3-PBA working solution to a concentration of 24.96 μg/l. The pool was shaken and divided into thirty 5-ml aliquots. Ten aliquots were analysed on the day of preparation to evaluate analytical reproducibility in pooled urine samples; the other twenty were stored in the freezer at −18°C to test stability (see Section 2.6).

Analytical reproducibility was also evaluated in different (individual) urine samples. Two 5-ml aliquots were taken from each of ten urine samples of subjects not occupationally exposed to pyrethroid insecticides; one was analysed as such and the other was spiked with the 3-PBA working solution to a concentration of 24.96 μg/l.

The reproducibility of the GC readings was evaluated by injecting one of the urine extracts obtained for the evaluation of analytical reproducibility ten times into the instrument.

To evaluate recovery, the results obtained during evaluation of analytical reproducibility in different (individual) urine samples were compared with those obtained with standard samples of 3-phenoxybenzoic acid (concentration of 24.96 μg/l) which had been derivatized only.

2.6. Stability of 3-phenoxybenzoic acid in urine samples stored at −18°C

Frozen samples were left at 4°C overnight and analysed in series of 3, 15, 30, 45, 60, 75 and 90 days after preparation.

2.7. Stability of 3-phenoxybenzoic acid to hydrolysis used for deconjugation

Six 5-ml aliquots of the same urine sample were spiked with the working solution of 3-phenoxybenzoic acid up to a concentration of 24.96 μg/l. Three of the aliquots underwent the complete analytical process, and the acid hydrolysis procedure was omitted for the others. Internal standard was added to the different aliquots after hydrolysis. The mean results of the two series of samples were compared to check for any decomposition of the metabolite during hydrolysis.

3. Results

Fig. 2 shows the chromatograms of urine samples derivatized and purified by our method with and without the addition of 3-PBA. Fig. 3 shows the chromatograms of urine samples derivatized by the method of Chester et al. [20] with and without 3-PBA, and purified by our method. The chromatograms are not only more complex, but there is also interference, near the 3-PBA peak, that cannot be resolved under our chromatographic conditions. In the method described by Chester et al. [20], the urine sample spiked with hydrochloric acid is maintained in reflux for an hour. After cooling, the urine is extracted with dichloromethane. The organic extract is concentrated and spiked with 1 ml of a 0.1 *M*

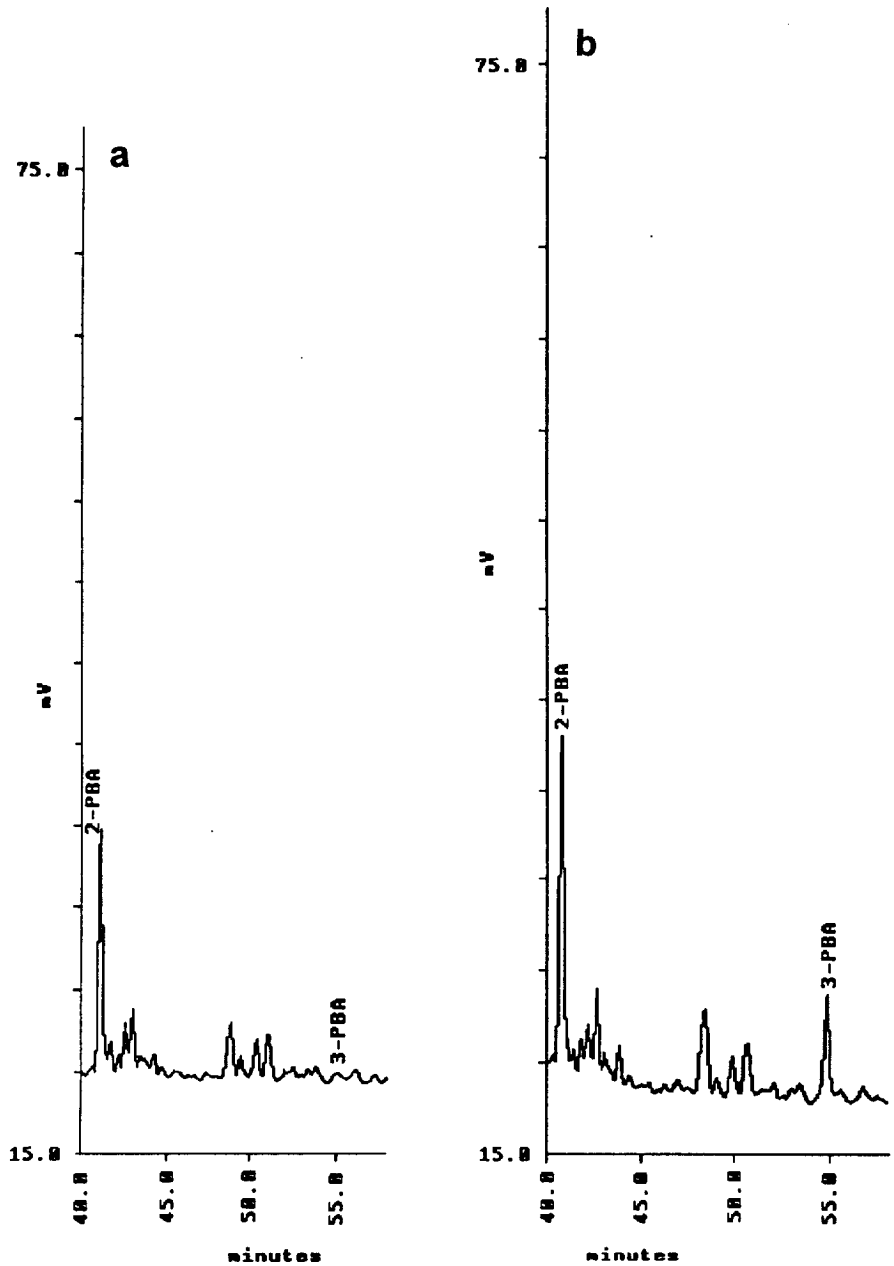

Fig. 2. Chromatograms of urinary extracts obtained by the present method of derivatization and purification: (a) urine blank; (b) urine spiked with 3-PBA to a concentration of 12.48 μg/l.

solution of tetrabutylammoniumphosphate in 0.2 *M* NaOH and 30 μl pentafluorobenzylbromide. The samples are shaken for 90 min and extracted with hexane. An aliquot of the organic phase is concentrated to 1 ml and deposited on an activated Florisil column (1 g) (heated to 105°C for 16 h). After deposition of the derivatized extract the column is washed with 10 ml hexane and 3-phenoxybenzoic

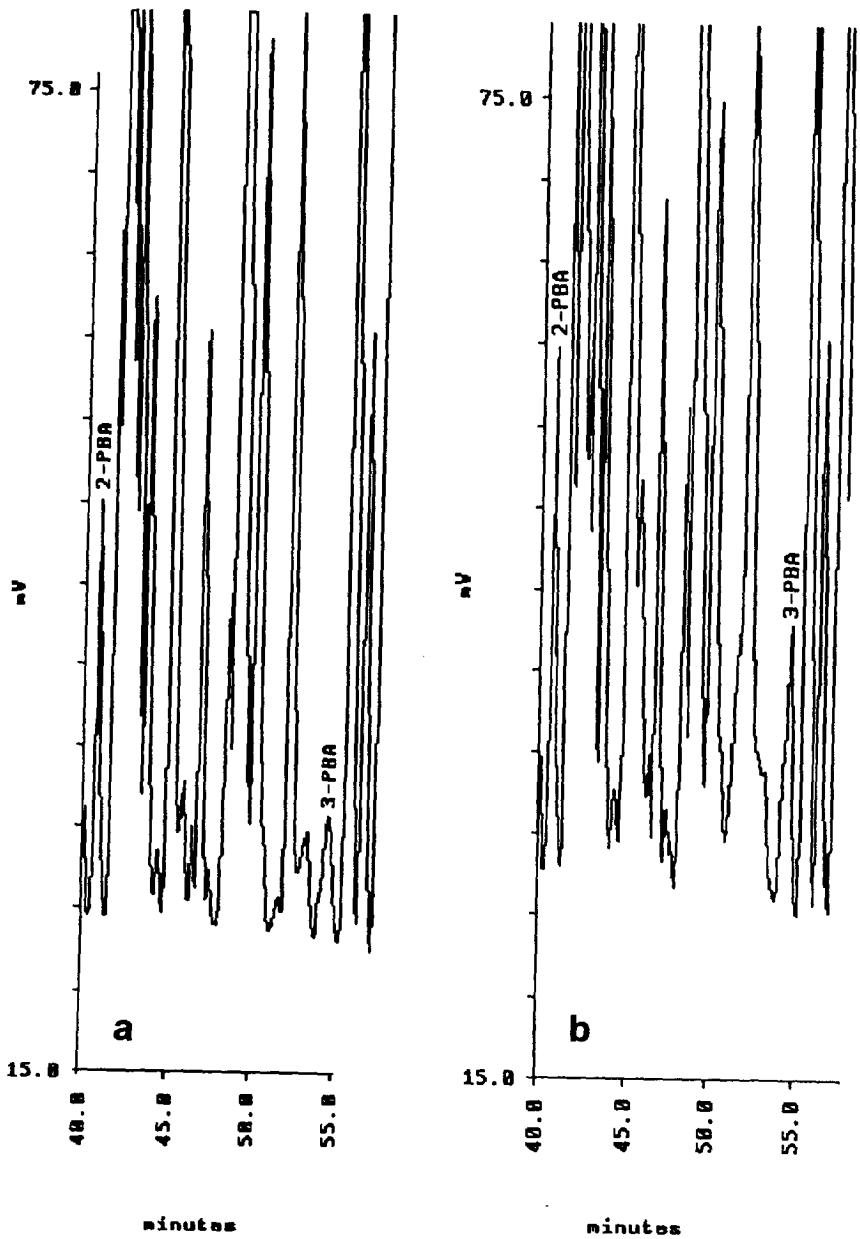

Fig. 3. Chromatograms of urinary extracts obtained by the derivatization procedure of Chester et al. [20] and the present purification method: (a) urine blank; (b) urine spiked with 3-PBA to a concentration of 12.48 μg/l.

acid is eluted with 25 ml of a mixture of ethylether (10%) and hexane. Analysis is performed by GLC–MS with a methyl silicone column.

Table 2 gives the precision, recovery and detection limit of the method based on ten determinations. The precision of the analytical method was similar for pooled (C.V.% 9.58) and different (individual) urine samples (C.V.% 10.34). In the latter case, analysis of the ten unspiked urine samples did not reveal detectable concentrations of the metabolite

Table 2
Recovery, detection limit and precision of the analytical method

	Mean recovery[a] (%)	C.V.1[b] (%)	C.V.1[c] (%)	C.V.2[d] (%)	Detection limit[e] (μg/l)
3-PBA	91.3 (90.9)	9.58	10.34	3.18	0.5

[a] Recovery evaluated in urine spiked with 3-PBA to a concentration of 24.96 μg/l. The value in parenthesis was obtained with the peak areas of 3-PBA without using the ratio with the internal standard.
[b] Coefficient of variation of the whole analysis on 10 aliquots of a pooled urine sample (3-PBA was added to the pooled sample to a concentration of 24.96 μg/l).
[c] Coefficient of variation of the whole analysis on 10 different urine samples (3-PBA was added to individual urine samples to a concentration of 24.96 μg/l).
[d] Coefficient of variation of chromatographic analysis only (10 replicates).
[e] The detection limit was calculated at a signal three times the background noise.

or interfering chromatographic peaks. The unpurified urine extracts could not be injected directly into the GC to evaluate recovery, due to the large number of interfering substances that prevent accurate detection of 3-PBA. We therefore compared the results obtained with urine (after purification) with those obtained with standard solutions at the same concentration which had only undergone derivatization. Mean recovery (91.3%) evaluated by comparing the ratios between the peak areas of 3-PBA and the internal standard in urine samples and standard solutions were not substantially different from that obtained comparing only the peak areas of 3-PBA (90.9%). This indicates that losses of analyte during extraction and purification did not exceed 10%. The detection limit of 0.5 μg/l was calculated on the basis of a signal three times the background noise. The chromatogram in Fig. 2b shows that the sample can be concentrated down to this detection limit.

Table 3 shows the results of the stability testing of 3-PBA in urine stored at −18°C. The concentrations of analyte did not vary significantly during 3 months of storage. This test was necessary as biological monitoring of exposure to pesticides is performed in certain periods of the year. Hundreds of samples reach the laboratory at the same time and obviously cannot all be analysed at once.

Table 3
Stability testing of urine samples spiked with 3-PBA to a concentration of 24.96 μg/l, stored for different times at −18°C

Storage (days)	Concentration of 3-PBA detected (μg/l)
0	24.90±2.39
15	24.98±1.89
30	26.02±2.41
45	25.87±2.52
60	24.43±2.11
75	23.64±2.62
90	25.85±1.32

Table 4 shows the results of the test carried out to evaluate the stability of 3-phenoxybenzoic acid during the hydrolysis procedure used for deconjugation. The results, expressed as ratio of peak area of 3-PBA to that of the internal standard, show that it made little difference whether or not hydrolysis was part of the analytical procedure. The internal standard was added to the various aliquots after hydrolysis in order to avoid any decomposition of 2-PBA that would change the above ratio.

Our method was tested for the assay of urinary 3-PBA in a subject exposed to esfenvalerate while preparing a mixture of the pesticide. The subject had the typical symptoms of synthetic pyrethroid poisoning. He excreted 3-PBA in the urine for four consecutive days after exposure. Creatinine was also assayed in urine samples of the subject monitored [21]. Concentrations decreased from 21.59 μg/g creatinine on the first day to 0.70 μg/g creatinine on the fourth day. The pattern of excretion is shown in Fig. 4 (unpublished data).

Table 4
Stability of 3-PBA added to urine to a concentration of 24.96 μg/l during the hydrolysis procedure used for deconjugation

Peak area 3-PBA/peak area 2-PBA without hydrolysis	Peak area 3-PBA/peak area 2-PBA with hydrolysis
0.87±0.07	0.86±0.09

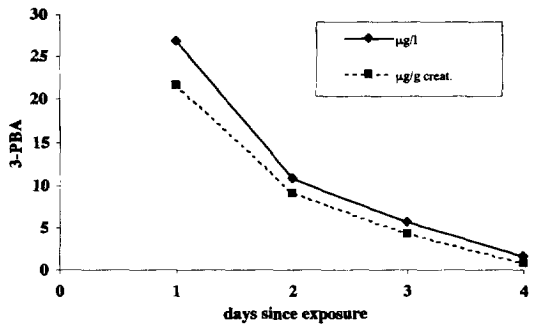

Fig. 4. Urinary excretion of 3-PBA in a worker engaged in mixing fenvalerate for spraying.

4. Discussion

Biological monitoring of exposure to pyrethroid insecticides is possible by assay of urinary 3-phenoxybenzoic acid, irrespective of whether a hydrogen of the benzyl group in the 3-phenoxybenzyl alcohol ester bond of the pyrethroid is substituted with a CN group. Two analytical methods [15,20] for the assay of this urinary metabolite are reported in the literature, and neither is suitable for routine use. GC with mass spectrometric detection [15,20] is a technique fairly widely used in laboratories but is less practical for routine investigations than the more common GC with electron-capture detection.

Derivatization with PFBBr is widely used for the assay of urinary metabolites of pesticides [22,23] to increase the volatility of the compounds and the response of detectors of the electron capture type. The derivatization of 3-phenoxybenzoic acid used by us is the same as that reported by Chau and Terry [24] for herbicides in standard solutions. Chau and Terry [24] propose derivatization times of 5 h or more to obtain maximum yield. Our method of leaving the sample to react overnight (about 15 h) has the advantage of not requiring supervision. Derivatization times are such that the whole analytical process cannot be carried out in a working day. With our method of derivatization, PFB esters of 3-PBA are formed at ambient temperature with a limited quantity of reagent, a well known for its tear compound. Wollen et al. [15] use anhydrous pentafluoropropionate as reagent with 1H,1H-pentafluoropropanol but the reaction must be run at 90°C.

Other methods of cold derivatization, like that proposed by Chester et al. [20] which uses 30 μl concentrated PFBBr with tetrabutylammonium phosphate in a NaOH environment, may be fast (90 min) but favour the formation of more interfering analytes that are not eliminated with our purification procedure.

Before derivatization, the urine is hydrolysed in the presence of concentrated sulphuric acid. This step is necessary to obtain a free form of the metabolite 3-PBA which is mostly excreted in conjugated form. Our hydrolysis procedure is the same as that reported by Wollen et al. [15]. Different hydrolysis procedures, such as the one proposed by Chester et al. [20] involving maintaining the urine sample in reflux in the presence of hydrochloric acid, have practical difficulties when large numbers of samples (15–20 samples) have to be processed at the same time.

It is necessary to purify the sample before injecting it into the chromatograph, otherwise one obtains an exceedingly large number of peaks, at least with detectors of the electron capture type. Without purification, the compounds of interest cannot be distinguished in the chromatogram because of interference, especially when their concentrations are low. Silica SPE columns were chosen for sample purification because they have a fixed phase that traps all high polarity compounds and can be used with a very apolar mobile phase (hexane–toluene) to elute the compounds of interest in an extremely selective manner. The Florisil columns used by Chester et al. [20] have the disadvantage of requiring manual preparation and of giving less reproducible results.

Purification of the sample is preceded by the addition of water to the reaction mixture and extraction with hexane. This is done to dissolve the analytes in an apolar solvent so that they can be purified in direct phase on silica SPE columns. Water separates the immiscible phases and destroys any residues of derivatizing agent. The residues form hydrobromic acid which is discarded with the aqueous phase.

Since no standards of the PFB esters of 3-phenoxybenzoic acid were available, the calibration curve was plotted using aliquots of a pool of urine spiked with standard 3-PBA and treated as samples of unknown titre. It is not necessary to know the yield of derivatization when using a calibration curve

plotted in this way for the determination of samples of unknown titre.

The detection limit of the present method (0.5 μg/l) was the same as that of Wollen et al. [15]. Chester et al. [20] report a detection limit of 1 μg/l for their method which required 50 ml urine and could hardly therefore be used for our purposes. Such large volumes of urine make handling lengthy and are not always available.

We can conclude that the present analytical method has excellent reliability (precision, detection limit, recovery) and is practical, safe and economical. Using 5 ml of sample and a routine assay technique it has the same precision and detection limits as methods using GC coupled with mass spectrometry.

Acknowledgments

The work was carried out in the Laboratory of Occupational Toxicology and Industrial Hygiene of the Institute of Occupational Medicine, University of Siena, Italy.

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