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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF FENVALERATE*

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SUMMARY

A simple and rapid analytical method is described for the detection and determination of the diastereoisomers of the synthetic pyrethroid, fenvalerate. It involves cleanup of the sample followed by chromatography on a μPorasil column in a high-performance liquid chromatograph using a solvent system of hexane–ethyl acetate (975:25, v/v) as the mobile phase. Under the conditions employed, the diastereoisomers were separated from one another within 12 min. The method was successfully employed to determine fenvalerate concentrations in hexane extracts of chicken excreta from a metabolic study of the insecticide. There was an indication that metabolites might be measured as well.

INTRODUCTION

Even though fenvalerate [(±)α-cyano-3-phenoxybenzyl-(±)2-(4-chlorophenyl)-3-methyl-1-butyrate] is not a cyclopropanecarboxylic acid ester, it is still considered a pyrethroid. It is a mixture of two diastereoisomers in a constant ratio. A variety of methods including thin-layer chromatography (TLC) and autoradiography[1,4,7,8], gas–liquid chromatography (GLC)[2,5,9,10], GLC–mass spectrometry (MS)[3], liquid scintillation radioanalysis[4], and high-performance liquid chromatography (HPLC)[6,9,11,12], have been reported for the study of this insecticide. Methods for TLC and autoradiography are time consuming and insensitive for the small quantities expected in metabolic and residue studies. Separation of the diastereoisomers in technical preparations has been achieved by the GLC techniques reported above. However, during GLC, thermal decomposition might occur which would impair quantitative estimation and yield metabolites similar or identical to those produced in normal metabolic processes.

In this respect, HPLC analysis at ambient temperature appears to be more suitable for metabolic studies of this synthetic pyrethroid. Earlier HPLC methods[6,9] that used a normal-phase system at ambient temperature and an ultraviolet (UV) detector separated the two diastereoisomers of fenvalerate. When an infrared (IR)

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detector was used[12], fenvalerate exhibited a single peak. An analytical method by HPLC was reported for the separation and determination of the four stereoisomers as their l-menthyl ester derivatives[11]. It is excellent for determination of the optical isomers in commercial preparations and thus efficacy as an insecticide. However, since high temperature is involved in the esterification technique, it does not lend itself to metabolic studies.

The method for HPLC analysis of fenvalerate at ambient temperature with a normal-phase system is presented in the following report as it was applied to a metabolic study of the synthetic pyrethroid in laying hens.

METHODS

Chemicals

Fenvalerate (Belmark®, Technical, 93.2% pure) was a generous gift of Shell Canada (Toronto, Canada). All solvents used for the mobile phase or in the extraction techniques were of HPLC-grade or pesticide-grade (Caledon Laboratories. Georgetown, Canada) and employed as supplied.

Chromatographic system

A Waters Assoc. Model 6000 high-performance liquid chromatograph equipped with a Waters Assoc. Model U6K loop injector and a Waters Assoc. Model 450 variable-wavelength UV detector was used. A 300 × 3.9 mm I.D. μPorasil liquid chromatography column was used and was protected by a 70 × 2.1 mm I.D. Whatman "guard column" packed with Co:Pell PAC. All separations were made at ambient room temperature and no attempt was made to control the temperature of the mobile phase or the column. Hexane (975 ml) and ethyl acetate (25 ml) were mixed as the isocratic mobile phase. Air bubbles in the mixture were removed using a combination of magnetic stirrer and aspirator.

Analytical method

Initially, a study was made of the relative UV absorption of the diastereoisomers of fenvalerate. Isocratic elution was carried out with the hexane–ethyl acetate mobile phase described above using a flow-rate of 1.2 ml/min; 5 μl (894.72 ng) of a solution of technical grade fenvalerate (Belmark) in hexane was injected; chromatograms were obtained over the range of 243 to 264 nm at intervals of 1 or 2 nm with a detector sensitivity of 0.02 a.u.f.s.; peak heights were used as a measurement of detector response.

This was followed by a study of the absorption of several different amounts of fenvalerate at 243, 254 and 264 nm. All other conditions remained identical while the amounts ranged from 504 ng to 5.317 μg. In order to test suitability of this method for analytical purposes, calibration curves were prepared which showed 1/2 peak height *versus* amount of fenvalerate.

Cleanup technique

Since a metabolic study of fenvalerate in laying hens involves examination of extracts of excreta, eggs and various organs and tissues, a cleanup technique was developed. Recovery of fenvalerate (101 μg) from SEP-PAK™ Florisil cartridges

(Waters Scientific, Mississauga, Canada) which had been washed with hexane (20 ml) was evaluated by elution with hexane–toluene (30:70, v/v). Each eluate (20 ml) was taken to dryness on a rotary flash evaporator at 65°C. The resultant residue was transferred with hexane (5 × 2 ml) into screw-capped graduated tubes (15 ml) and taken to dryness again at ambient temperature under a gentle-stream of dry nitrogen gas. The residue was made up in hexane to a final volume of 1.0 ml from which suitable aliquots (5, 25 μl) were taken for HPLC injection using identical conditions to those outlined above.

Recovery of fenvalerate from chicken excreta

A sample of fresh chicken excreta (1.5 kg) was thoroughly mixed in a Waring blender and divided into two equal portions. To one portion, sufficient fenvalerate in solution in a small volume of acetone was added to make 50 ppm and to the other sufficient was added to make 100 ppm. The individual portions were thoroughly remixed in a Waring blender. Each portion was further divided into 2 equal parts, one of which was analyzed directly while the other was freeze-dried prior to analysis.

Extraction from fresh excreta. Several aliquots (10 g) of the "spiked" fresh excreta samples were placed in glass-stoppered erlenmeyer flasks (50 ml). Methanol (20 ml) was added to each flask and the contents shaken vigorously for 1 h (Lab-Line Junior Orbit Shaker). The mixtures were filtered carefully through Whatman No. 1 paper into round-bottom flasks (100 ml). The erlenmeyer flasks were washed with methanol (5 × 2 ml) and the washings added to the round-bottom flasks. The filter paper and residues were replaced in the erlenmeyer flasks and reextracted with methanol (20 ml) as above. The combined methanol extracts were taken to dryness on a rotary flash evaporator at 35°C.

Extracted residue with hexane (6 × 2 ml) and placed clear coloured extracts into screw-capped centrifuge tubes (15 ml). Extracts were taken to dryness by means of a gentle stream of dry nitrogen at ambient temperature. Repeated hexane extraction of residues from methanol extracts once more. Reduced volume of hexane extracts to 1 ml with the aid of dry nitrogen at ambient temperature. These entire extracts were subjected to cleanup as described above and just prior to HPLC analysis, an appropriate dilution of 0.1 to 1.0 ml was made with hexane so that a 5-μl injection would contain amounts of fenvalerate in the useful measurable range.

Extraction from freeze-dried excreta. Several aliquots (2 g) of the freeze-dried "spiked" excreta samples were placed in hexane-washed extraction thimbles in Soxhlet apparatus and extracted with hexane (150 ml) for 7 h using round-bottom flasks (300 ml). In another trial, the extraction was carried out overnight (16 h). The extracts were taken to dryness on a rotary flash evaporator at 30°C. The resultant residue was redissolved in hexane (5 × 2 ml) and transferred to screw-capped centrifuge tubes (15 ml). The hexane was reduced in volume (1 ml) by means of a gentle stream of dry nitrogen at ambient temperature. The entire coloured extracts were subjected to cleanup by the method outlined above. Just prior to HPLC analysis, an appropriate dilution, 0.1 to 1.0 ml with hexane, was made so that a 5-μl injection would contain amounts of fenvalerate in the useful measurable range.

Technique applied to a metabolic study in laying hens

Excreta was collected individually on a daily basis from 2 groups of Single

Comb White Leghorns, each of 6 hens at >80% production which had been fed *ad libitum* a standard growing ration containing either 50 or 100 ppm fenvalerate and which had each received a daily oral dose of ^{14}C-fenvalerate, a mixture of carbonyl- and phenoxy-labeled compounds, in Risella oil (Shell) —0.108 μCi/0.5 ml, $2.4 \cdot 10^4$ dpm, for 7 days. The excreta samples were freeze-dried, ground thoroughly in a Wiley mill and stored at 4°C in screw-capped glass jars until analysis.

Duplicate aliquots (2 g) were extracted with hexane (150 ml) for 7 h by the Soxhlet technique followed by methanol (150 ml) for 16 h. Each extract was taken to dryness on a rotary flash evaporator, redissolved in its respective solvent and transferred to centrifuge tubes (15 ml). The volumes were reduced to 2 ml by means of a gentle stream of nitrogen at ambient temperatures. Aliquots (0.1 ml) were taken for radioactivity measurements by combustion and scintillation counting. The remaining extract from each hen was pooled by group on a daily basis and reduced in volume to 10 ml under a flow of nitrogen at ambient temperature. Several aliquots (1 ml) were subjected to cleanup and HPLC analysis by the techniques described herein.

RESULTS AND DISCUSSION

In the normal-phase system employed, replicate injections resulted in decreased retention times and a slightly poorer separation of the diastereoisomers during a single day's operation. Insertion of the "guard column" between the injection loop

TABLE I

RELATIVE UV ABSORPTION OF DIASTEREOISOMERS OF FENVALERATE

HPLC conditions: column, μPorasil (300 × 3.9 mm I.D.); mobile phase, hexane–ethyl acetate (975:25, v/v) at a flow-rate of 1.2 ml/min; detector sensitivity, 0.02 a.u.f.s.; injection volume, 5 μl (894.72 ng) of a solution of fenvalerate in hexane.

Wave length (nm)	*Early peak* 1/2 peak height (cm)*	*Later peak** 1/2 peak height (cm)*
243	5.5	4.6
245	4.4	3.5
252	2.6	2.1
253	2.4	1.9
254	2.1	1.7
255	2.0	1.6
256	1.8	1.5
257	1.6	1.4
258	1.7	1.3
259	1.4	1.1
260	1.5	1.1
261	1.3	1.2
262	1.2	0.9
263	1.2	1.0
264	1.2	1.0

* Under the conditions described the retention time of the early peak was 9.27 ± 0.23 (S.E.M.) min.
** The retention time of the later peak was 10.25 ± 0.24 (S.E.M.) min.

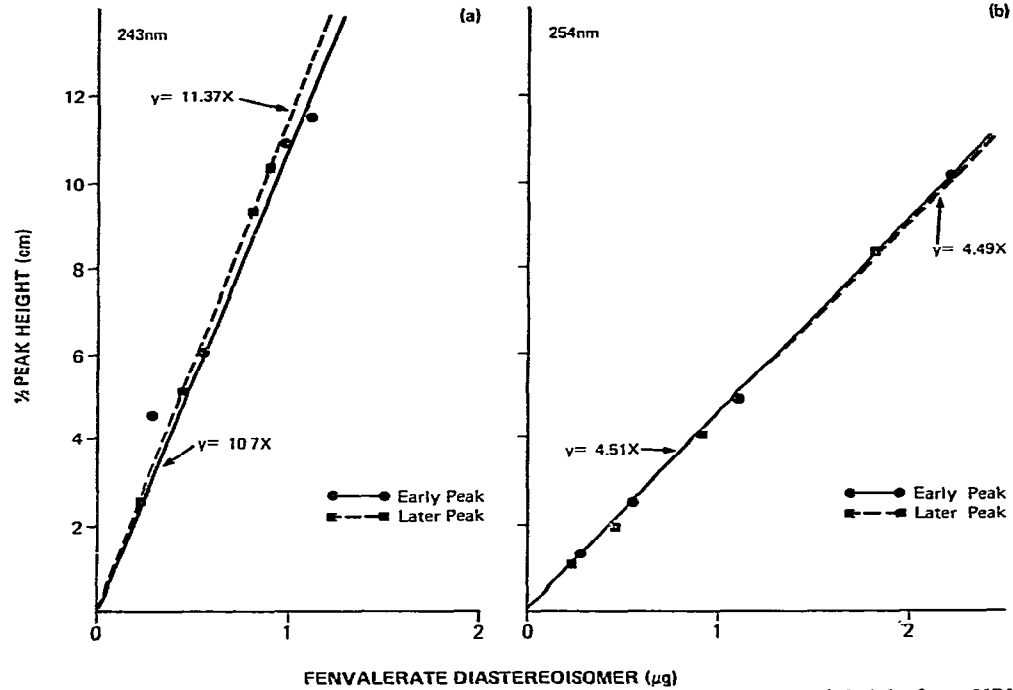

Fig. 1. Linear plots of amounts of diastereoisomers of fenvalerate *versus* 1/2 peak height from HPLC on μPorasil column of 5-μl aliquots of hexane solutions prepared by serial dilution of fenvalerate standard (69.3 mg/10 ml). Mobile phase, hexane–ethyl acetate (975:25, v/v); flow-rate, 1.2 ml/min; detector at (a) 243 nm, (b) 254 nm; sensitivity, 0.02 a.u.f.s.; chart speed, 0.5 cm/min.

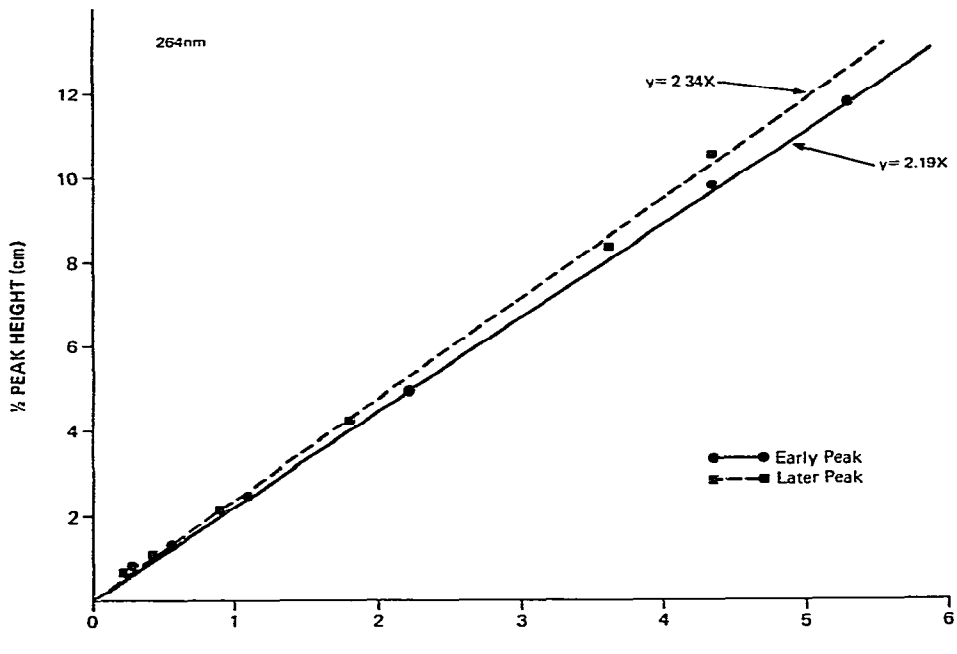

Fig. 2. Linear plots of amounts of diastereoisomers of fenvalerate *versus* 1/2 peak height from HPLC on μPorasil column of 5-μl aliquots of hexane solutions prepared by serial dilution of fenvalerate standard (69.3 mg/10 ml). Mobile phase, hexane–ethyl acetate (975:25, v/v); flow-rate, 1.2 ml/min; detector at 264 nm; sensitivity, 0.02 a.u.f.s.; chart speed, 0.5 cm/min.

and the μPorasil column prevented this from happening and extended the life of the latter column to 3 or 4 weeks before it required regeneration in the recommended (Waters Assoc.) manner.

The data for the relative UV absorption study are shown in Table I. Under the HPLC conditions described, the diastereoisomeric mixture of technical fenvalerate was separated into two diastereoisomers, an early eluting peak with a retention time of 9.27 ± 0.23 min (mean ± s.e.m.) and a later eluting peak with a retention time of 10.25 ± 0.24 min. As was expected, the greatest relative absorption as measured by 1/2 peak height was 243 nm. However, if substances which interfere with absorption at that wavelength are present in extracts under examination, any of the wavelengths up to 264 nm would be satisfactory if the amount of fenvalerate was about 900 ng.

The data in Figs. 1 and 2 indicate that a straight line function exists between amount of fenvalerate and absorption expressed as 1/2 peak height for each of the three wavelengths investigated. At 243 nm the useful measurable amount of fenvalerate falls in the 250–1000-ng range; at 254 nm, in the 250–2250-ng range; and at 264 nm, in the 250–5000-ng range.

The recovery of fenvalerate from SEP-PAK Florisil cartridges by elution

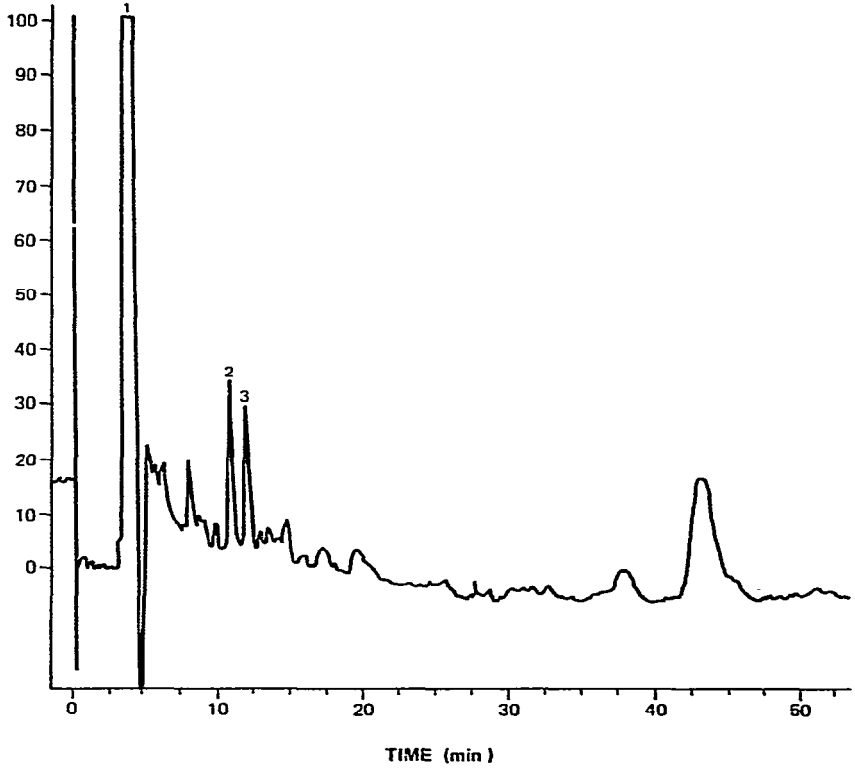

Fig. 3. HPLC on μPorasil column of 25-μl aliquot, prior to cleanup, of pooled hexane extracts of chicken excreta from a metabolic study of fenvalerate. Mobile phase, hexane–ethyl acetate (975:25, v/v); flow-rate, 1.2 ml/min; detector at 243 nm; sensitivity, 0.02 a.u.f.s.; chart speed, 0.5 cm/min. Peaks: 1 = injection artefact; 2, 3 = diastereoisomers of fenvalerate.

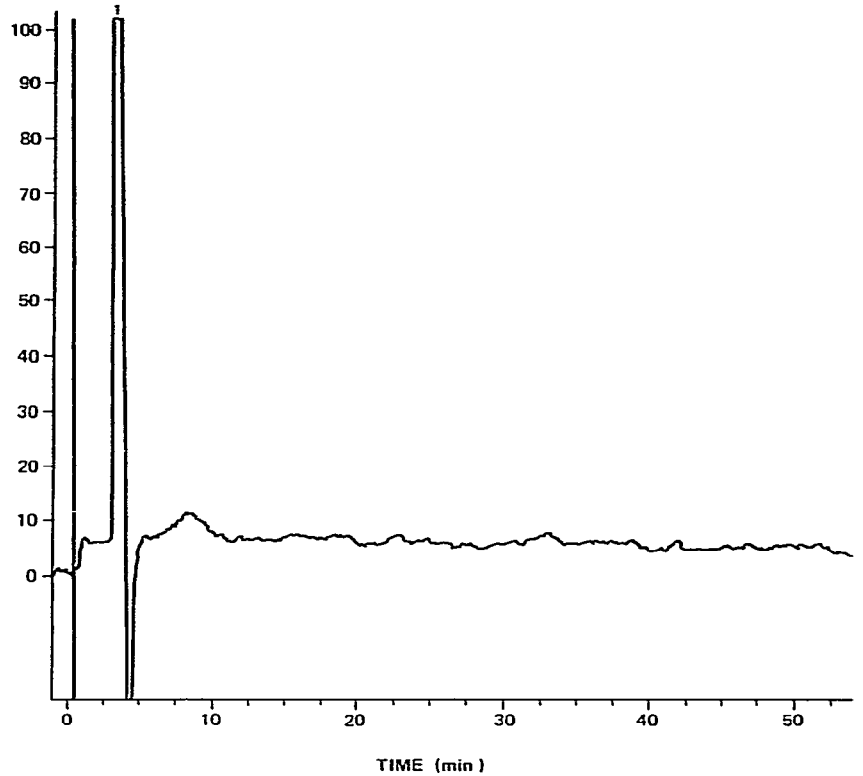

Fig. 4. HPLC on μPorasil column of 25-μl aliquot of hexane eluate of pooled hexane extract of chicken excreta from SEP-PAK Florisil cartridge during cleanup technique. Mobile phase, hexane–ethyl acetate (975:25, v/v); flow-rate, 1.2 ml/min; detector at 243 nm; sensitivity, 0.02 a.u.f.s.; chart speed, 0.5 cm/min. Peak: 1 = injection artefact.

with hexane–toluene (30:70, v/v) as described above was 98.3 to 100.3%. No fenvalerate could be detected in other than the first eluate (20 ml).

The recovery of fenvalerate from 50 ppm "spiked" fresh chicken excreta was 105.2 ± 4.7% (100.5–109.8%) and from 100 ppm "spiked" fresh chicken excreta was 98.1 ± 5.8% (92.3–103.9%).

The recovery from freeze-dried 50 ppm "spiked" chicken excreta was 90.7 ± 2.5% (83.6–96.3%) and from freeze-dried 100 ppm "spiked" chicken excreta was 88.7 ± 2.8% (79.9–96.1%). There was no significant difference between a 7-h and a 16-h extraction. Similarly, a further 7-h or 16-h Soxhlet extraction with fresh hexane (150 ml) did not recover additional fenvalerate.

The data indicate that for residues up to 100 ppm, quantitative recovery is obtainable by extraction and analysis of fresh chicken excreta by the methods described herein. However, it is often more convenient to freeze-dry excreta samples in order to store them prior to analysis. The data indicate that this technique results in some fenvalerate loss; only approximately 90% recovery is obtained. This phenomenon has been noted, in this laboratory, in previous analyses for radioactivity in excreta from fenvalerate-fed laying hens which had also received oral doses of ^{14}C-

fenvalerate. Some of the radioactivity remains bound to the residual excreta after extraction with hexane followed by methanol. The amount of radioactivity remaining can be confirmed by combustion and conversion to carbon dioxide. The type of binding is currently unknown but is under investigation.

Fig. 3 is a tracing of a chromatogram which resulted from HPLC analysis, prior to cleanup, of 25 μl of pooled hexane extracts of chicken excreta from a metabolic study of fenvalerate. Figs. 4 and 5 are tracings of chromatograms of eluates from the SEP-PAK cleanup technique. Prior to cleanup, the hexane extracts of chicken excreta are highly colored. This color remains on the SEP-PAK cartridge. In Fig. 3, in addition to the injection artefact, several other peaks, including those for the diastereoisomers of fenvalerate, are evident. Some or all of them may be due to metabolites of fenvalerate and their identities are currently under investigation. Fig. 4 indicates that, during the placement of the sample in hexane on the SEP-PAK cartridge for cleanup, no fenvalerate or other compounds were eluted in the limited volume of hexane which was employed. Fig. 5 indicates that a single 60-ml eluate of hexane–toluene (30:70, v/v) contains the diastereoisomers of fenvalerate and the earlier, less polar peaks. However, all the more polar peaks remained on the cartridge. It

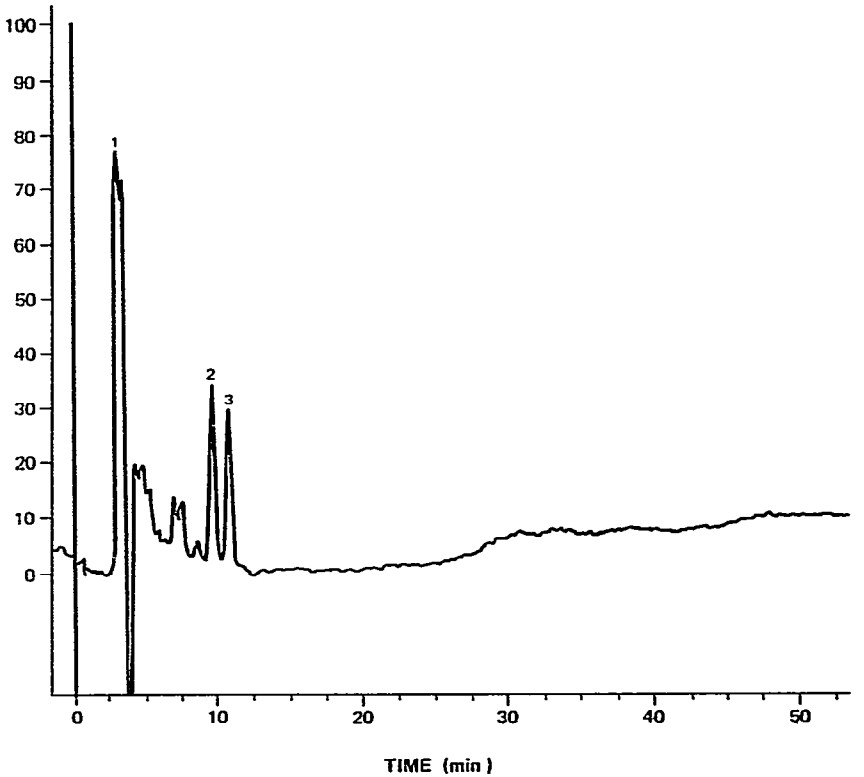

Fig. 5. HPLC on μPorasil column of 25-μl aliquot of hexane–toluene (30:70, v/v) eluate of pooled hexane extract of chicken excreta from SEP-PAK Florisil cartridge during cleanup technique. Mobile phase, hexane–ethyl acetate (975:25, v/v); flow-rate, 1.2 ml/min; detector at 243 nm; sensitivity, 0.02 a.u.f.s.; chart speed, 0.5 cm/min. Peaks: 1 = injection artefact; 2, 3 = diastereoisomers of fenvalerate.

is evident that if a standard fenvalerate sample has been run just prior to or just after the unknown sample, the separation of the diastereoisomers is such that determination of the amount of fenvalerate is no problem.

The methods described are being used to analyze all the excreta samples from the metabolic study. Identification of metabolites by combined chromatographic and spectroscopic techniques is anticipated. In addition, quantitative analysis of these metabolites should be possible by HPLC.

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REFERENCES

1 H. Ohkawa, K. Nambu, H. Inui and J. Miyamoto, *J. Pesticide Sci.*, 3 (1978) 129–141.
2 Y. W. Lee, N. D. Westcott and R. A. Reichle, *J. Ass. Offic. Anal. Chem.*, 61 (1978) 869–871.
3 R. L. Holmstead, D. G. Fullmer and L. O. Ruzo, *J. Agr. Food Chem.*, 26 (1978) 954–959.
4 H. Ohkawa, H. Kaneko, H. Tsuli and J. Miyamoto, *J. Pesticide Sci.*, 4 (1979) 143–155.
5 I. H. Williams and M. J. Brown, *J. Agr. Food Chem.*, 27 (1979) 130–132.
6 D. Mourot, B. Delépine, J. Boisseau and G. Gayot, *J. Chromatogr.*, 168 (1979) 277–279.
7 H. Ohkawa, K. Nambu and J. Miyamoto, *J. Pesticide Sci.*, 5 (1980) 215–223.
8 N. Mikami, N. Takahashi, K. Hayashi and J. Miyamoto, *J. Pesticide Sci.*, 5 (1980) 225–236.
9 M. Horiba, K. Kitahara, K. Takahashi, S. Yamamoto and A. Murano, *Agr. Biol. Chem.*, 44 (1980) 1197–1199.
10 P. G. Wszolek, D. H. Lein and D. J. Lisk, *Bull. Environ. Contam. Toxicol.*, 24 (1980) 296–298.
11 M. Horiba, H. Kitahara, S. Yamamoto and A. Murano, *Agr. Biol. Chem.*, 44 (1980) 1361–1365.
12 E. Papadopoulou-Mourkido, Y. Iwata and F. A. Gunther, *J. Agr. Food Chem.*, 28 (1980) 1043–1049.