Journal of Chromatography, 461 (1989) 337–346
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1428

DETERMINATION OF PYRETHROID RESIDUES ON PADDY RICE BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

PAUL R. HADDAD*, JOHN G. BRAYAN, GERARD J. SHARP and SERGIO DILLI
Department of Analytical Chemistry, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033 (Australia)
and
JAMES M. DESMARCHELIER
CSIRO Division of Entomology, P.O. Box 1700, Canberra, A.C.T. 2601 (Australia)

SUMMARY

Several synthetic pyrethroids and the synergist piperonyl butoxide have been determined as aged residues on paddy rice by reversed-phase high-performance liquid chromatography with detection at 225 nm. These compounds are commonly used as protectants for stored grains. Studies on the comparative rates of extraction of both the pesticides and interfering material from the grain were conducted with acetone, methanol, and hexane as extracting solvents. Acetone was the best of these solvents because it provided quantitative extraction of the pesticides over a 48-h period, and did not give high levels of ballast material. Pyrethroids present in the extract at levels in excess of 0.5 μg/ml could be determined by direct injection, but at lower concentrations, clean-up and preconcentration were required. Clean-up of acetone extracts was accomplished with either Florisil or alumina pre-columns, and up to a tenfold preconcentration was achieved by adsorption of the pesticide on a C_{18} pre-column, or by concentrating the extract through evaporation of the solvent. These approaches gave good recoveries and linear calibration plots. Detection limits were of the order of 0.05 μg/ml.

INTRODUCTION

Pyrethroid insecticides, such as bioresmethrin, phenothrin, fenvalerate, permethrin, and deltamethrin, together with the synergist piperonyl butoxide, are in current use as grain protectants. Since these pyrethroids are much more expensive than organophosphate pesticides, the levels at which they are applied are kept as low as possible. A commonly encountered situation is the application of a small amount of a pyrethroid in conjunction with a larger concentration of an organophosphate pesticide. This method is particularly suitable for the control of a specific pest that shows resistance to the organophosphate[1,2]. For example, 0.5–1 mg/kg of bioresmethrin can be applied to wheat with 10 mg/kg of fenitrothion, together with 5–10

0021-9673/89/$03.50

mg/kg of piperonyl butoxide, which acts a synergist increasing pesticide activity without having any insecticidal properties of its own.

It has been established[3–8] that polar solvents, such as methanol, are the most suitable for the extraction of aged residues of carbaryl and organophosphates from grain. Here, an aged residue refers to a pesticide that has been in contact with the grain for a substantial period of time. Little work has been performed on extraction of pyrethroids from grain, but it has been suggested that non-polar solvents may be useful for this purpose[4]. In this paper, studies are reported on the extraction of aged pyrethroid residues from grain with acetone, methanol, or hexane.

Reversed-phase high-performance liquid chromatography (HPLC) has been applied to the determination of piperonyl butoxide on a number of grains[9], and to the determination of pyrethroid residues on barley and wheat[10,11]. Fluorescence detection was used for piperonyl butoxide and UV absorption detection for the pyrethroids. A normal-phase HPLC method has also been reported for the determination of bioresmethrin on wheat[12]. In a previous communication[13], we have described the use of reversed-phase HPLC for the analysis of carbaryl and organoposphate pesticides, and in this paper we report a simple method for the analysis of pyrethroids and piperonyl butoxide on paddy rice for the purpose of monitoring rates of pesticide decay under controlled storage conditions. The concentrations of pesticide considered in this work are therefore those at which the efficacy of the pesticide is retained, and in all of the rice samples considered, the identities of the pesticides applied were known.

EXPERIMENTAL

Instrumentation

The liquid chromatograph consisted of Millipore Waters (Milford, MA, U.S.A.) Model 510 and 501 pumps, a Model 481 variable-wavelength detector, and Model 740 data module. The column was a Waters Novapak C_{18} stainless-steel column (150 × 3.9 mm I.D.), equipped with a Waters Guard Pak pre-column module. A Rheodyne (Cotati, CA, U.S.A.) 7000 six-port switching valve was used during the preconcentration step.

Reagents

Bioresmethrin, phenothrin, permethrin, deltamethrin, fenvalerate, and piperonyl butoxide standards were obtained from the Curator of Standards, Australian Government Analytical Laboratories (Melbourne, Australia). The solvents used were HPLC-grade methanol and acetonitrile and Nanograde hexane and acetone (Mallinkrodt, Oakleigh, Australia). Florisil Sep-Paks, obtained from Millipore, and basic alumina from Ajax (Sydney, Australia) were used for the clean-up of extracts.

The paddy rice, treated with phenothrin and permethrin, was obtained from a storage facility at Home Hill in Queensland, Australia. The rice was treated with pesticide as it was loaded into the silo on a conveyor belt, as is the normal practice in the industry. The pesticides were applied at a dosage rate of approximately 1 mg/kg, and the rice was stored for six months before extraction. The other pesticides used in this study were applied in the laboratory, *ca.* two months prior to analysis. In this case the pesticides were dissolved in 2 ml of acetone, and this was applied dropwise to 1 kg of rice in a large plastic bag. The application levels were 2 mg/kg for deltamethrin and 10 mg/kg for bioresmethrin, fenvalerate, and piperonyl butoxide.

Extraction

Extraction studies were performed by mixing 30 g of whole rice containing an aged pesticide with 50 ml of solvent in a stoppered conical flask and allowing the mixture to stand with occasional manual shaking. Each extraction was carried out in triplicate. For rice treated with permethrin, phenothrin, deltamethrin, and piperonyl butoxide, 1-ml aliquots of the extract were taken after 1, 4, 25, 48, and 72 h, whereas for the remaining pesticides, aliquots were taken after 48 and 72 h only. Comparative extraction studies were conducted on rice samples containing permethrin and phenothrin by either ginding the grain in a blender, followed by extraction with a solvent for 48 h, as described above, or by subjecting the whole grain to Soxhlet extraction for 8 h.

Sample clean-up

The following two clean-up methods were used.

(i) An aliquot (1 ml) of the extract was transferred to a small test tube and evaporated to near dryness, under a stream of nitrogen. The remaining few drops were shaken twice with 1 ml of hexane. With the aid of a syringe, the combined hexane phase was passed through a Florisil (Sep-Pak) cartridge, followed by 3 ml of acetone–hexane (15:85, v/v). Both eluates were collected and evaporated to dryness under a stream of nitrogen. Finally, the residue was dissolved in 1 ml of methanol for later analysis.

(ii) A small alumina column was made by plugging a Pasteur pipette with cotton and adding 0.5 g of basic alumina. Extract (1 ml) was then passed through the column, followed by 1 ml of pure acetone. The combined eluates were evaporated to 1 ml under a stream of nitrogen.

Analysis

Extracts containing pesticides at concentrations exceeding 0.5 μg/ml could be analysed by direct injection without clean-up. A suitable volume (10 μl) of extract was injected into the column and eluted with a mobile phase of 75% aq. acetonitrile at a flow-rate of 1 ml/min. For detection an absorption wavelength of 225 nm was used. Methanol and acetone extracts could be injected directly, but is was necessary to evaporate hexane extracts to dryness under a stream of nitrogen and to redissolve the residue in 1 ml of methanol prior to injection. The pesticides were identified in the chromatograms obtained by comparison of retention times with those of standards, and by confirmatory analysis using capillary gas chromatography with electron-capture or flame-ionization detection.

When the concentration of pesticide in the extract was less than 0.5 μg/ml, a sample preconcentration step was necessary. Two different preconcentration methods were used.

(i) Solid-phase extraction. This was process was accomplished with the aid of a six-port high-pressure switching valve, using the instrumental configuration shown in Fig. 1. The sample extract was first treated by the Florisil clean-up method described above, and 100 ml of the purified extract was injected into a C_{18} pre-column (Waters Guard Pak), using 40% aq. acetonitrile as the mobile phase. After 30 s, the mobile phase was changed to 75% aq. acetonitrile by rotating the switching valve, and the pyrethroid was passed into the analytical column. Both pumps were operated at a flow-rate of 1 ml/min.

(ii) Evaporation. Extract (10 ml) was evaporated to dryness in a rotary evaporator, and the residue was redissolved in 1–2 ml of acetone and purified by the alumina method described above.

RESULTS AND DISCUSSION

Extraction of aged residues

Three solvents, acetone, methanol, and hexane, were evaluated to determine which provided the most efficient extraction of the pesticides. The results for the extraction studies are shown in Table I, from which it can be seen that acetone and methanol generally extracted 10–15% more pyrethroid and about 50% more piperonyl butoxide than hexane. Grinding the grain or the use of Soxhlet extraction did not increase the levels of pesticide extracted. To investigate possible losses of pesticide from hexane extracts during the evaporation and redissolution steps necessary before injection, two equivalent series of pesticide standards were made up in methanol and hexane extracts of rice which had not been treated with a pesticide. The methanol extracts were injected directly onto the column, whilst the hexane extracts were evaporated to dryness and redissolved in methanol prior to injection. The results

TABLE I

EXTRACTION OF PESTICIDES FROM WHOLE GRAIN BY METHANOL, ACETONE AND HEXANE

Pesticide	*Approx. application rate (mg/kg)*	*Extraction time (h)*	*Amount extracted (mg/kg)*		
			Methanol	*Acetone*	*Hexane*
Permethrin	1	1	1.0	0.8	0.8
		4	1.1	1.0	0.8
		24	1.3	1.1	1.1
		48	1.3	1.1	1.2
		72	1.2	1.1	1.1
Phenothrin	1	1	0.7	0.7	0.5
		4	0.8	0.7	0.6
		24	1.0	1.0	0.9
		48	1.0	1.1	0.9
		72	1.1	1.1	0.9
Deltamethrin	2	1	2.0	1.8	1.3
		4	2.3	2.3	1.6
		24	2.3	2.3	2.2
		48	2.5	2.5	2.2
		72	2.5	2.5	2.2
Bioresmethrin	10	48	8.0	8.6	7.0
		72	8.2	8.5	6.7
Fenvalerate	10	48	7.8	9.0	7.3
		72	8.2	8.6	7.2
Piperonyl butoxide	10	1	5.8	4.8	3.8
		4	8.2	7.8	4.6
		24	11.2	10.6	5.9
		48	12.2	10.8	6.7
		72	11.5	10.7	7.2

TABLE II
RECOVERIES OBTAINED FOR THE FLORISIL AND ALUMINA CLEAN-UP PROCEDURES

Pesticide	*Concentration (μg/ml)*	*Recovery (%)*	
		Florisil clean-up	*Alumina clean-up*
Fenvalerate	1.0	96	100
Phenothrin	1.0	86	105
Bioresmethrin	1.0	84	100
Permethrin	1.0	88	105
Deltamethrin	1.0	83	100
Piperonyl butoxide	10.0	90	105

obtained by both methods were equivalent, indicating that no significant losses of pesticides from the hexane extracts had occurred.

Acetone was selected as the most suitable solvent for the extraction of pyrethroids from paddy rice, because the level of ballast material was much lower for this solvent than with methanol. The optimal extraction period was 48 h.

Sample clean-up

A Florisil clean-up procedure had been developed for the analysis of organophosphate pesticides in rice extracts[13] and was shown to give acceptable recoveries for typical organophosphates (*e.g.* 90 ± 7% for fenitrothion). This approach was found to be applicable to the pyrethroids, and Table II shows that recoveries in excess of 83% were obtained with this method. The optimal eluent was acetone–hexane (3:17), since this solvent eluted the pesticides but minimised the level of interfering material eluted from the Sep-Pak. Subsequent studies showed that much of the polar material extracted from rice by acetone, which ultimately interfered with the reversed-phase HPLC analysis of pesticides, could be removed by passing the extract through a column of basic alumina. Although this method was not always as effective as the Florisil clean-up, in many cases it was sufficient, and the alumina adsorbent showed no affinity for the pesticides, as indicated by the quantitative recoveries shown in Table II.

Analytical procedure

Calibration data and detection limits for the pyrethroids and piperonyl butoxide, injected at 225 nm without clean-up or preconcentration, are given in Table III. The detection limit was defined as the concentration of pesticide in a 10-μl injection which produced a signal-to-noise ratio of 3. Although the pesticides studied show stronger absorption at wavelengths below 225 nm, analysis at these wavelengths was impractical due to the presence of strongly absorbing contaminants. Under the chromatographic conditions described, all of the pyrethroids were eluted with retention times in the range 10–15 min, and piperonyl butoxide was eluted after about 5 min, as shown in Table IV. The *cis*- and *trans*-isomers of permethrin and phenothrin were separated. When the pesticide levels in the extract were greater than 0.5 μg/ml, analysis without clean-up was possible, but in some cases interference by extractives with piperonyl butoxide was observed. This could be prevented by changing the wavelength to 237 nm for the elution of piperonyl butoxide.

TABLE III

CALIBRATION DATA AND DETECTION LIMITS FOR DIRECT INJECTION OF PESTICIDES WITHOUT SAMPLE CLEAN-UP

Pesticide	*Working calibration range** (*μg/ml*)	*Detection limit*	
		On rice (*mg/kg*)	*In extract* (*μg/kg*)
Fenvalerate	0.4–4	0.7	0.4
Phenothrin	1–4	1.0	0.6
Bioresmethrin	0.4–4	0.7	0.4
Permethrin	1–4	1.0	0.6
Deltamethrin	0.4–4	0.7	0.4
Piperonyl butoxide	4–40	1.7	1.0

* Correlation coefficients of 0.998 or higher were obtained for each of the stated ranges.

The pyrethroids are applied to grain at levels as low as 0.5 mg/kg, and the described extraction procedure would therefore produce concentrations of pesticide of *ca.* 0.3 μg/ml in the final extract. For this reason, preconcentration of the extract was necessary, and this was achieved by solid-phase extraction of the pesticides on a C_{18} pre-column. The sample extract containing pyrethroids was loaded onto a C_{18} pre-column conditioned with 40% aq. acetonitrile, and the bound pesticides were subsequently passed into the analytical column using 75% acetonitrile (Fig 1). The preconcentration procedure described under Experimental provided a ten-fold sample concentration factor. Table V shows calibration data for the preconcentration method with Florisil clean-up, and Fig. 2 compares the chromatograms obtained by direct injection and preconcentration of an extract which had been spiked with bioresmethrin, piperonyl butoxide, and the organophosphate fenitrothion. The extracts were subjected to Florisil clean-up prior to preconcentration in order to remove some of the extractives which accumulated on the pre-column. However, considerable amounts of early-eluted interfering material were still present (Fig. 2b), and this would limit adaptation of the preconcentration approach for on-line clean-up, unless further steps

TABLE IV

RETENTION TIMES OF PYRETHROIDS

Mobile phase, 75% aq. acetonitrile; flow-rate, 1 ml/min; Novapak C_{18} column.

Pesticide	*Retention time* (*min*)
Piperonyl butoxide	5.5
Deltamethrin	11.5
Bioresmethrin	11.5
Fenvalerate	12.2
trans-Permethrin	13.5
cis-Phenothrin	14.4
trans-Phenothrin	15.6
cis-Permethrin	16.2

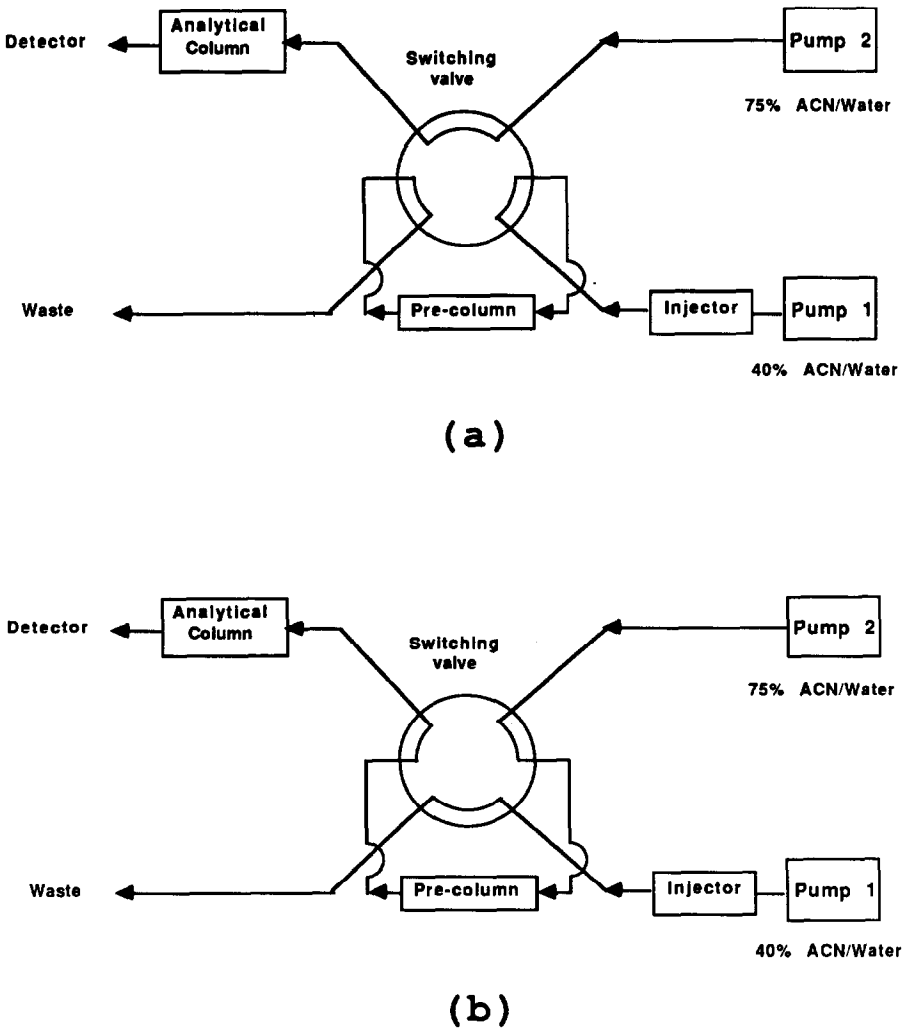

Fig 1. Schematic representation of the solid-phase preconcentration procedure, showing (a) loading of the sample and (b) transfer of the bound pesticide to the analytical column. ACN = acetonitrile.

were incorporated to clean the pre-column periodically. The chromatographic conditions employed in the preconcentration method were selected because they provided the best overall separation of all the pyrethroids and piperonyl butoxide. Further optimization of this approach could yield cleaner chromatograms for more limited mixtures.

One disadvantage of the above method was the requirement for an extra pump and switching valve, and changes in the tubing would be necessary if the HPLC was to be used for normal operation. Therefore, studies were undertaken in which the sample clean-up was combined with preconcentration by evaporation of the solvent. This was achieved by concentrating the sample from 10 ml to 1 ml in a rotary evaporator, and

TABLE V

CALIBRATION DATA AND DETECTION LIMITS FOR PESTICIDES AFTER CLEAN-UP AND PRECONCENTRATION

Preconcentration method	*Clean-up procedure*	*Pesticide*	*Working calibration range* (μg/ml)*	*Detection limit*	
				On rice (mg/kg)	*In extract (μg/ml)*
Solid-phase	Florisil	Fenvalerate	0.1–1	0.07	0.04
		Phenothrin	0.1–1	0.12	0.07
		Bioresmethrin	0.1–1	0.07	0.04
		Permethrin	0.1–1	0.07	0.04
		Deltamethrin	0.1–1	0.12	0.07
Evaporation	Alumina	Fenvalerate	0.06–0.6	0.07	0.04
		Phenothrin	0.1–0.6	0.17	0.1
		Permethrin	0.1–0.6	0.17	0.1
		Deltamethrin	0.06–0.6	0.07	0.04

* Correlation coefficients of 0.991 or higher were obtained for each of the stated ranges.

subsequent clean-up by the Florisil or alumina methods. The latter approach was preferred because of its simplicity. Calibration data and detection limits obtained by this method are listed in Table V. Piperonyl butoxide and bioresmethrin are not included, since early-eluted extractives made it difficult to quantitate the former pesticide, and the latter was volatilised to some extent during the evaporation step. Fig. 3 shows chromatograms obtained by injection of 2 μg/ml deltamethrin in an acetone extract of rice without preconcentration or clean-up (Fig. 3a), and an injection of 0.2 μg/ml deltamethrin after preconcentration and clean-up (Fig. 3b). These chromatograms show that quantitative preconcentration had been achieved, without undue interference by other extracted materials.

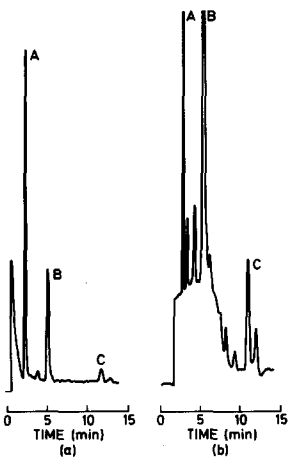

Fig. 2. Chromatograms of pyrethroids after Florisil clean-up, followed by (a) direct injection or (b) solid-phase preconcentration. Peaks: A = 5 μg/ml fenitrothion, B = 8 μg/ml piperonyl butoxide, C = 0.5 μg/ml bioresmethrin. Injection volumes: (a) 10 μl, (b) 100 μl; mobile phase, 75% aq. acetonitrile; flow-rate, 1.0 ml/min; detection, 225 nm.

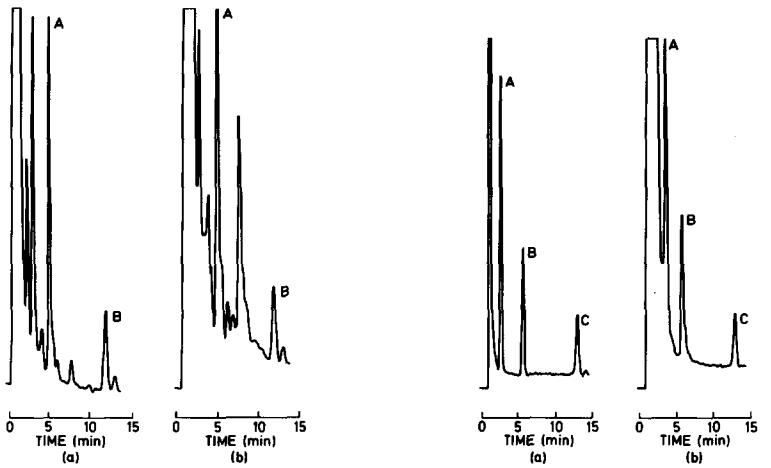

Fig. 3. Chromatograms of a spiked rice extract (a) without clean-up and (b) after preconcentration by solvent evaporation, followed by alumina clean-up. Sample: (a) 10 μl of extract, containing 20 μg/ml piperonyl butoxide (A) and 2 μg/ml deltamethrin (B): (b) 10 μl of extract, containing 2 μg/ml piperonyl butoxide (A) and 0.2 μg/ml deltamethrin (B), after evaporation, preconcentration, and alumina clean-up. Chromatographic conditions as for Fig. 2.

Fig. 4. Chromatograms of spiked rice extract (a) after and (b) before Florisil clean-up. Sample: 10 μl of extract, containing 5 μg/ml fenitrothion (A), 8 μg/ml piperonyl butoxide (B), and 2 μg/ml bioresmethrin (C). Chromatographic conditions as for Fig 2.

Both of the above preconcentration methods are limited to ten-fold preconcentration factors, because of the levels of interfering extractives present in acetone extracts, even after clean-up. In the evaporation method, a small peak appeared which was eluted close to the *trans*-permethrin and *trans*-phenothrin isomers, and in both methods a late-eluted, broad peak occasionally emerged and caused interference with subsequent analyses. This problem could be avoided by washing the column with 85% aq. acetonitrile for 5 min after every five or six injections. More extensive clean-up procedures would undoubtedly improve the detection limits and provide cleaner chromatograms, but the methods developed are adequate for grain screening purposes and have the advantage of being relatively rapid and straightforward.

Multiresidue analysis

As mentioned previously, pyrethroids are often applied to grain in conjunction with piperonyl butoxide and an organoposphate pesticide. Simultaneous analysis of these three types of compounds is possible, but clean-up is required for accurate quantitation. This is illustrated in Fig. 4, which shows the effect of Florisil clean-up on the determination of piperonyl butoxide, bioresmethrin and the organophosphate fenitrothion, in an acetone extract of rice. The relatively polar nature of the organophosphates enables them to be easily separated from the pyrethroids and piperonyl butoxide on a reversed-phase column. However, one organophosphate, pirimiphos methyl, was found to be inseparable from piperonyl butoxide under the chromatographic conditions used. The organophosphate pesticides and piperonyl butoxide are applied at levels that do not require preconcentration for their

determination. When concentration was necessary for pyrethroid analysis, it proved to be more reliable to analyse the extract for piperonyl butoxide and the organophosphate before the preconcentration step, because of the large number of early-eluted interfering materials present in the concentrated extracts (Figs. 2 and 3).

CONCLUSIONS

The method described is a simple technique for the analysis of organophosphates, piperonyl butoxide, and pyrethroids on paddy rice by reversed-phase HPLC. Altough this method lacks the sensitivity of gas chromatography with electron-capture detection, it permits the analysis of all pyrethroids, as well as piperonyl butoxide and organophosphate pesticides, in the concentration ranges likely to occur in stored grain.

ACKNOWLEDGEMENT

Financial assistance from the Australian Centre for International Agricultural Research (ACIAR) is gratefully acknowledged.

REFERENCES

1 J. M. Desmarchelier, *J. Stored Prod. Res.*, 13 (1977) 129.
2 M. Bengston, R. A. H. Davies, J. M. Desmarchelier, R. Hennig, W. Murray, B. W. Simpson, J. T. Snelson, R. Sticka and B. E. Wallbank, *Pestic. Sci.*, 14 (1983) 373.
3 J. M. Desmarchelier, M. Bengston, M. Connell, W. Minett, B. Moore, M. Phillps, J. Snelson, R. Sticka and K. Tucker, *Pestic. Sci.*, 8 (1977) 473.
4 J. M. Desmarchelier, *J. Pestic. Sci.*, 5 (1980) 521.
5 M. C. Bowman, M. Beroza and D. B. Leuck, *J. Agric. Food Chem.*, 16 (1968) 796.
6 P. A. Hargreaves and K. J. Melksham, *Pestic. Sci.*, 14 (1983) 347.
7 R. T. Krause, *J. Agric. Food Chem.*, 26 (1978) 1333.
8 R. T. Krause, *J. Assoc. Off. Anal. Chem.*, 63 (1980) 1114.
9 K. Isshiki, S. Tsumura and T. Watanabe, *Bull. Environ. Contam. Toxicol.*, 19 (1978) 518.
10 P. Bottomley and P. G. Baker, *Analyst (London)*, 109 (1984) 85.
11 R. M. Noble, D. J. Hamilton and W. J. Osborne, *Pestic. Sci.*, 13 1982) 246.
12 D. S. Gunew, in G. Zweig (Editor), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. 10, Academic Press, New York, 1978, p. 19.
13 J. G. Brayan, P. R. Haddad, G. J. Sharp, S. Dilli and J. M. Desmarchelier,*J. Chromatogr.*, 447 (1988) 249.