Journal of Chromatography, 447 (1988) 249–255
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 619

Note

Determination of organophosphate pesticides and carbaryl on paddy rice by reversed-phase high-performance liquid chromatography

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(First received March 3rd, 1988; revised manuscript received May 2nd, 1988)

Carbaryl and organophosphate pesticides such as fenitrothion are frequently applied as protectants to stored grain. However, whereas the organophosphates are generally used alone, they may also be applied in conjunction with the carbamate insecticide carbaryl for the control of a specific pest showing resistance to the organophosphate. Since, in the case of stored grain, pesticides are usually applied at levels between 5 and 10 mg/kg (ref. 1) and the pesticides decay with time, there is the need to establish that, on the one hand, the grain is not consumed when residue levels are still high and, on the other, that the risk of infestation by insects is not overly increased when the levels become too low. Through loss of pesticide and its efficacy, both dependent upon the relative humidity and the temperature used for storage of the grain, the concentration where the pesticides become ineffective falls typically in the range of 1–2 mg/kg. Thus, for quality control purposes in the monitoring of pesticide levels on stored grain, the concentration range of interest is 1–10 mg/kg.

Although reversed-phase high-performance liquid chromatography (HPLC) has often been used for the analysis of carbaryl on grain[2–4], this is not the case for the organophosphate pesticides[5]. Such a situation can be attributed to the fact that carbaryl is difficult to analyse by gas chromatography because of thermal instability, and that HPLC detectors often lack the sensitivity required for organophosphate analysis. Nevertheless, the pesticide concentration in extracts of such grains is within the working sensitivity range of the UV detector so that there is no reason why the same HPLC column cannot be used for the analysis of both carbaryl and organophosphate residues to provide a simple and accurate method for monitoring the levels of these pesticides in grains.

In designing an analytical procedure, previous work[2,6,7] has suggested that methanol is the most suitable solvent for the extraction of "aged" residues* from grain. In addition, methanol extracts are suitable for direct injection onto columns employed in reversed-phase HPLC. With all of these factors in mind, this paper describes the development of a suitable method for the simultaneous determination

* This term is used to refer to a pesticide which has been in contact with the grain for some time.

0021-9673/88/$03.50

of the carbamate pesticide carbaryl and a group of organophosphate pesticides in methanol extracts of rice, using reversed-phase HPLC. The organophosphates chosen for this study were the compounds fenitrothion, pirimiphos-methyl, chlorpyrifos-methyl, methacrifos and etrimfos and are currently in use, or under development for use, as grain protectants.

EXPERIMENTAL

Instrumentation

The liquid chromatograph consisted of Millipore Waters (Milford, MA, U.S.A.) Model 510 and 501 pumps, Model 660 solvent programmer, Model 481 variable-wavelength UV detector and Model 740 data module. The column was a Waters reversed-phase Nova-Pak C_{18} stainless-steel column (150 mm long × 3.9 mm I.D.), equipped with a Waters Guard-Pak pre-column module.

The instrument used for obtaining UV-absorption spectra was a Varian (Sunnyvale, CA, U.S.A.) Series 634 spectrophotometer. The spectra were measured over the wavelength range 200–300 nm, using a solution of the pure pesticide dissolved in methanol.

Reagents

Pure carbaryl, methacrifos, fenitrothion, chlorpyrifos-methyl, etrimfos and pirmiphos-methyl were obtained from the Curator of Standards, Australian Government Analytical Labaratories (Melbourne, Australia). The solvents used in this work were HPLC-grade methanol, Nanograde hexane and acetone (Mallinckrodt, Oakleigh, Australia) and HPLC-grade acetonitrile (Ajax, Auburn, Australia). Florisil cartridges ("Sep-Pak", from Millipore, Bedord, MA, U.S.A.) were used for the clean-up of crude extracts of the grain used in this study.

Analytical procedure

The pesticides were extracted from the grain by mixing 30 g of rice with 50 ml of methanol in a stoppered conical flask and allowing the mixture to stand for 48 h with occasional manual shaking. The following clean-up procedure was then used. An aliquot (1.0 ml) of the methanol extract was transferred to a small test-tube and evaporated to near-dryness, using a stream of nitrogen. The remaining few drops,

TABLE I

CHROMATOGRAPHIC CONDITIONS FOR PESTICIDE ANALYSIS

Pesticide	*Mobile phase (% acetonitrile in water)*	*Retention time (min)*	*Detection wavelength (nm)*
Carbaryl	40	6.2	225
Methacrifos	50	5.9	225
Fenitrothion	60	5.5	267
Etrimfos	60	8.9	225
Chlorpyrifos-methyl	60	11.5	225
Pirimiphos-methyl	65	8.5	247

consisting mainly of water extracted from the rice, were then shaken twice with hexane (1 ml) and, with the aid of a syringe, the combined hexane phase was carefully passed through a Florisil (Sep-Pak) cartridge, followed by 3 ml of acetone–hexane (40:60). Both eluates were collected and evaporated to dryness, using a stream of nitrogen. Finally, the residue was dissolved in pure methanol (1.0 ml) for later analysis.

For HPLC analysis, 10 μl of sample were injected onto the column using a mobile phase of acetonitrile–water under isocratic or gradient conditions and a flow-rate of 1 ml/min. The percentage of acetonitrile and the detection wavelength used were varied according to the particular pesticides being analysed, and individual conditions are listed in Table I.

RESULTS AND DISCUSSION

Selection of chromatographic conditions

From UV-absorption spectra for each of the pesticides, carbaryl and methacrifos showed maximum absorption at 222 nm and 220 nm, respectively, whilst pirimiphos-methyl absorbed most strongly at 247 nm. For the remaining pesticides maximum absorption occurred at about 205 nm, with fenitrothion exhibiting a further absorption band at 267 nm. However, since methanol was found to extract a considerable amount of coloured and UV-absorbing material from rice, it was im-

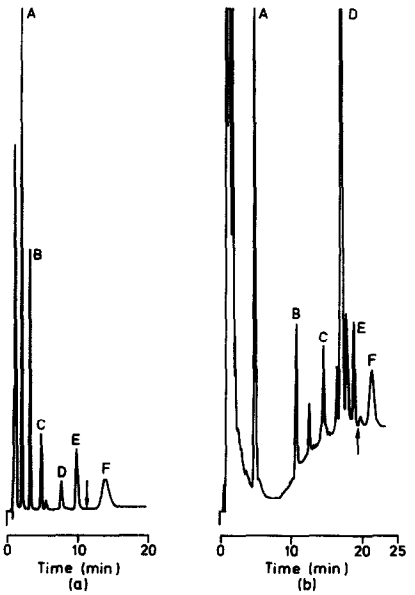

Fig. 1. Chromatograms showing the simultaneous separation of carbaryl and five organophosphates standards by isocratic elution (a) and in a methanol extract of rice by gradient elution (b). Mobile phases: (a) acetonitrile–water (60:40), and (b) gradient of acetonitrile–water (40:60) to (70:30) over 12 min. Analytical wavelength: initially 225 nm, then changed to 247 nm after 11 min (a) or 19.5 min (b), as indicated by arrows. Solute concentrations: 4 μg/ml. Peaks: A = carbaryl; B = methacrifos; C = fenitrothion; D = etrimfos; E = chlorpyrifos-methyl; F = pirimiphos-methyl.

practical to attempt detection at 205 nm. For this reason, an analytical wavelength of 225 nm was used for all pesticides, with the exception of fenitrothion and pirimiphos-methyl which were detected at 267 nm and 247 nm, respectively. Even with detection at 225 nm, direct injection of the crude extracts produced unsatisfactory chromatograms because of the number of peaks present. However, direct analysis of these extracts was possible by choosing more appropriate chromatographic conditions. Table I lists the optimal conditions which achieved resolution of each of the pesticides from the coextractives in the shortest time. It is noteworthy that even slight changes in mobile phase composition caused considerable variation to measured retention times.

Separation of all six pesticides in a single run was also possible provided the wavelength was changed to aid detection of pirimiphos-methyl (see Fig. 1a). Under the same conditions, all of the pesticides could not be fully resolved from the coextractives when using a spiked extract. However, as shown in Fig. 1b, it was possible to use gradient elution (40–70% acetonitrile over 12 min) for the determination of pesticides in untreated extracts, except in the case of etrimfos which experienced excessive interference from the co-extracted material.

Sample clean-up

Whilst manipulation of the detection wavelength was partially successful in minimising interferences from co-extracted material, it was predictable that this measure would result in some loss of sensitivity. Moreover, since the amount of coextractives from rice was dependent on both the type of rice and the area in which it was grown, a clean-up procedure became an essential part of the analytical procedure.

It is significant that most of the material extracted from the grain by methanol

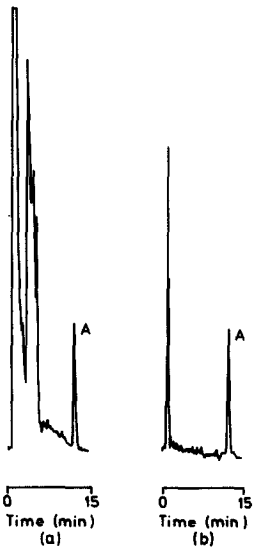

Fig. 2. Chromatograms of chlorpyrifos-methyl (A) in a methanol extract of rice before (a) and after (b) sample clean-up. Solute concentration: 6 μg/ml. Other conditions as in Table I.

TABLE II

CALIBRATION DATA AND DETECTION LIMITS FOR PESTICIDES

Pesticide	*Concentration range in extracts (μg/ml)*	*Correlation coefficients of calibration plots*	*Recovery (%) ± S.D. (n=4)*	*Detection limits* (mg/kg)*
Carbaryl**	2–10	0.999		
	0.2–1.5	0.999	97 ± 3	0.05
Methacrifos**	2–15	0.999		
	0.2–1.5	0.999	100 ± 1	0.2
Fenitrothion	2–15	0.999	90 ± 7	
	0.3–1.5	0.999	98 ± 5	0.4
Etrimfos	2–15	0.992	97 ± 9	
	0.8–2.0	0.992	95 ± 5	1.0
Chlorpyrifos-methyl	1–10	0.998	91 ± 6	
	0.4–1.0	0.999	90 ± 8	0.6
Pirimiphos-methyl	2–15	0.999	93 ± 4	
	0.2–1.5	1.000	85 ± 3	0.3

* Calculated for the sample of rice from the detection limits of the pesticide in the methanol extract after 30 g of grain were treated with 50 ml of solvents as under *Analytical procedure* in the Experimental section.

** For this pesticide, no clean-up of the sample extract was used.

was relatively polar and was readily separated from the less polar pesticides. In fact, this separation was readily accomplished on a Florisil ("Sep-Pak") cartridge, where the pesticides were eluted with acetone–hexane but the co-extractives were retained by the adsorbent. The effect of this clean-up is shown in Fig. 2 for rice extracts treated with chlorpyrifos-methyl, before and after sample clean-up. Recoveries for this procedure, as shown in Table II, ranged from 90–100% for all pesticides except for methacrifos (70%) and carbaryl (30%), which provided quantitative recoveries without sample clean-up. The poor recoveries for these two species when the clean-up procedure was applied may be attributable to the fact that some methacrifos, being a volatile compound, may have been lost during the evaporation stage of the clean-up process, whilst the rather polar carbaryl may not be extracted quantitatively into hexane. Fortunately, since both of these pesticides absorb strongly at 225 nm, they are relatively easy to quantify without cleanup, as shown in Fig. 3.

Since the clean-up method produced superior chromatograms when compared with direct injection of grain extracts, this method is clearly preferable for the determination of low concentrations of pesticides, or for multi-residue methods where more than one pesticide may be present. However, clean-up is time-consuming and for laboratories which have a large sample throughput and require rapid results (such as those operated by grain-handling authorities), the direct injection of extracts may be adequate. Here, it may be noted that multiple injections of unclean extracts do not appear to have any adverse effects on the life-time or efficiency of the analytical column, provided that a guard column is also employed.

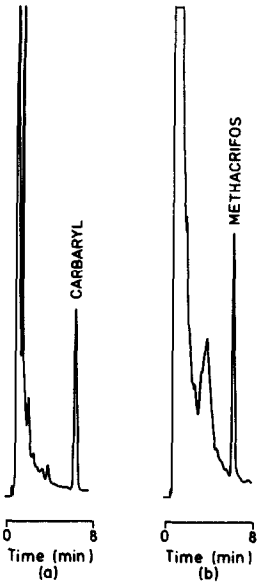

Fig. 3. Chromatograms for carbaryl (5 μg/ml) (a) and methacrifos (10 μg/ml) (b), in a methanol extract of rice, without clean-up. Mobile phases: acetonitrile–water (40:60) for carbaryl and acetonitrile–water (50:50) for methacrifos. Detection at 225 nm.

Detection limits

Table II summarizes the detection limits for each of the pesticides used in this study, as well as the correlation coefficients for the linearity of two series of standards added to rice extracts. The data shown are for samples which had been treated using the proposed clean-up procedure, except in the cases of carbaryl and methacrifos which were determined in the untreated methanol extracts, that is, without the clean-up step. The standard additions were prepared to cover the range of pesticide levels likely to be found in stored grain and where possible (depending on the individual detection limits), one order of magnitude below this. Thus the range 2–15 μg/ml in the injected solution corresponded to 3.3–25 mg/kg in the rice sample when the outlined extraction procedure was used. The detection limits quoted are defined as the concentration of pesticide in the original rice sample giving a detector response of three times the baseline noise. Since the pesticides were well separated from the peaks due to co-extractives, the detection limits for clean and unclean extracts were very similar. It is obvious from the data that good linear calibration plots were obtained for the two concentration ranges chosen.

Applications

In using the analytical procedure discussed, the conditions specified in Table I are suitable for the analysis of individual pesticides. For samples where more than one pesticide is present, separation is possible using isocratic conditions following clean-up of the sample extract, or gradient elution with the conditions discussed earlier. When analysis of extracts containing carbaryl and an organophosphate is required, clean-up is not feasible because of the poor recoveries of carbaryl. In such

cases, rather than using the gradient elution mode, it was found to be more expedient to analyse all of the samples for carbaryl using acetonitrile–water (40:60) as the mobile phase, and then to change the conditions and re-analyse for the organophosphate pesticide.

For multiresidue analysis, where the identities of the applied pesticides are unknown, sample clean-up is necessary, followed by isocratic analysis using the conditions given in Fig. 1. Although full recovery of carbaryl and methacrifos is not obtained with the clean-up procedure, the presence of these pesticides would be revealed so that the untreated extract could then be re-examined under the optimal conditions for the determination of these species.

CONCLUSION

Despite the relative lack of sensitivity for the analysis of organophosphate residues, reversed-phase HPLC does offer some advantages for the determination of grain protectants on rice. First, direct injection of grain extracts is possible without the problems of non-volatile co-extractives which can interfere with gas chromatographic analysis. Second, polar extraction solvents such as methanol can be employed, and third, both carbaryl and organophosphate insecticides can be analysed wihtout changing the column or detector. The sample clean-up procedure described is straightforward, uses minimal materials and is applicable to most of the pesticides studied. This clean-up procedure provides a method for the analysis of samples containing two pesticides, or for the screening of multiresidue samples.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support of the Australian Centre for International Agricultural Research (ACIAR).

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