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EXTRACTION AND LIQUID–SOLID CHROMATOGRAPHY CLEANUP PROCEDURES FOR THE DIRECT ANALYSIS OF FOUR PYRETHROID INSECTICIDES IN CROPS BY GAS–LIQUID CHROMATOGRAPHY*

RALPH A. CHAPMAN and CAROL R. HARRIS

Agriculture Canada Research Institute, University Sub Post Office, London, Ontario N6A 5B7 (Canada)

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SUMMARY

The extraction of four pyrethroid insecticides, fenpropanate (WL 41706, S-3206), permethrin (WL 43479, FMC-33297, PP 557, NRDC-143), cypermethrin (WL 43467, NRDC 149), and fenvalerate (WL 43775, S-5602) from carrots, tomatoes celery, and onions with acetone followed by extraction of the resulting acetone–water mixture with hexane, was investigated and found suitable for residue analysis. The elution of the four materials from two types of silica and alumina adsorbents with mixtures of organic solvents of varying polarity was examined. The elution patterns and recoveries from silica gel, aluminum oxide, and water deactivated Florisil indicated potential utility in cleanup. Florisil was found to provide cleanup for asparagus, carrots, tomatoes, tobacco, and onions permitting analyses to < 0.01 ppm for most of the insecticides by electron capture gas–liquid chromatography, at extract concentrations of 1 g/ml. The other adsorbents, singly or in combination, were found to provide the cleanup required for radishes and those cases involving other crops where Florisil cleanup was insufficient.

INTRODUCTION

The new pyrethroid insecticides are the object of intense interest for use in crop protection because their toxicological properties permit control of certain insect species at application rates as low as 70–140 g/hectare. Commercial introduction will depend in part on the availability of residue methodology for crops of interest. At least four of these compounds are easily analyzed by gas–liquid chromatography (GLC) because of their thermal stability and electron capturing properties[1]. The non-specific nature of the electron capture detector (ECD) introduces the further requirement that methodology be developed to provide interference free crop extracts. Suitable cleanup procedures for the GLC analyses of permethrin on green peas and cabbage[2] and on potatoes[3], as well as fenvalerate on cabbage[4] using EC detection have

* Contribution No. 722, Research Institute, London, Ontario N6A 5B7, Canada.

been reported. We wish to report our observations on the extraction of fenpropanate (WL 41706, S-3206), permethrin (NRDC-143, WL 43479, FMC-33297, ICI PP557), cypermethrin (NRDC-149, WL 43467), and fenvalerate (WL 43775, S-5602) from crop material, their elution from Florisil, silica gel, aluminium oxide, and activated alumina and the utility of these procedures for analysis in asparagus, carrots, tomatoes, tobacco, onions, and radishes by GLC–ECD.

MATERIALS AND METHODS

Chemicals and apparatus

Permethrin [3-phenoxybenzyl (±)-*cis,trans*-3(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] (93.9%) was supplied by Chipman Chemicals (Stoney Creek, Canada). Fenpropanate [(±)-alpha-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate] (99%), cypermethrin [(±)-alpha-cyano-3-phenoxybenzyl (±) *cis,trans*-3(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (96%) and fenvalerate [(±)-alpha-cyano-3-phenoxybenzyl (±)-2-(4-chlorophenyl)-3-methyl butyrate] (97%) were provided by Shell Research (Woodstock Agricultural Research Centre, Sittingbourne, Great Britain).

Hexane (Code No. 641100, Shell, Oakville, Canada), benzene (ACS reagent; Fisher Scientific, Toronto, Canada) chloroform and acetone (ACS reagent, Caledon Labs., Georgetown, Canada) were distilled in glass in our laboratory, the hexane and benzene from potassium permanganate and sodium–lead alloy (dri-Na, J. T. Baker, Phillipsburgh, N.J., U.S.A.) and the chloroform and acetone from permanganate only. Persons using the procedures described should be aware that the effects of these solvents on human physiological processes have not been fully examined and appropriate care should be taken to keep exposure to an absolute minimum until the effects are carefully evaluated.

Florisil (60–100 mesh, normally activated by manufacturer) (Floridin Co., Pittsburgh, Pa., U.S.A.), silica gel (100–200 mesh, Grade 923) (Fisher), aluminium oxide (reagent powder) and alumina (80–200 mesh, activated, chromatographic grade Alcoa® type F-20) (Matheson, Coleman & Bell, East Rutherford, N.J., U.S.A.) contained 2.4, 4.2, 0.7, and 1.5%, moisture (24 h at 110°).

Chromatography columns were 1.5 cm I.D. glass fitted with a coarse fritted glass disk at the bottom and a ground glass joint at the top to permit attachment of a 250-ml reservoir.

Crop samples were from untreated control plots at our field station. The equipment and conditions for analysis have been described previously[1].

Extraction and fortification

Fresh plant material was reduced to roughly 1:1 acetone–water extract by macerating a suitable weight of chopped sample with acetone (1 ml/g) in a blender for 2 min at 20,000 rpm. The macerate was filtered with suction using a Büchner funnel and Whatman No. 1 paper. The blender jar was rinsed with acetone (*ca.* 0.3 ml/g) which was added to the residue in the funnel only after most of the initial filtrate had been collected. The rinse was allowed to begin to seep through without suction before the vacuum was reapplied. The filtrate was quantitatively transferred to a separatory funnel with acetone rinses, hexane was added (0.5 ml/g) and the mixture was diluted

TABLE I

RECOVERY OF INSECTICIDES FROM CROPS

Results given as: upper 0.5 ppm level/lower 0.05 ppm level.

Crop	*Extraction*	*Recovery (%)*			
		Permethrin	*Fenpropanate*	*Cypermethrin*	*Fenvalerate*
Carrot	1	64/57	81/72	85/67	82/70
	2	10/17	13/19	13/15	11/20
	Total	74/74	94/91	98/82	93/90
Tomato	1	74/69	78/78	76/65	80/83
	2	18/9	9/8	17/7	19/12
	Total	92/78	87/86	93/72	99/95
Celery	1	82/73	81/76	80/71	86/83
	2	14/10	7/7	11/7	12/11
	Total	96/83	88/83	91/78	98/94
Onion	1	63/—*	86/—*	78/69	74/74
	2	8/—	7/—	4/30	7/26
	Total	71/—	93/—	82/99	81/100

* Unable to determine 0.05 ppm accurately because of interference in onions used for this fortification (see text).

with an equal volume of water. Starting in this manner the acetone–water mixture was extracted twice with hexane (0.5 ml/g) which was transferred to a storage bottle. Anhydrous sodium sulfate was added to the extract which was stored at freezer temperature until used for analysis. Samples were fortified with the insecticides by adding suitable amounts dissolved in acetone at both the maceration and acetone-water filtrate stages. Pyrethroids added to the filtrate at a level equivalent to 0.5 ppm in the crop were 90–100% recovered in all the crops tested. Recoveries of the equivalent of 0.5 and 0.05 ppm from the maceration step are given in Table I. The second extraction refers to the maceration and subsequent extraction of the plant fiber remaining in the Büchner funnel after the first extraction.

Liquid–solid chromatography

Columns packed (bottom upward) with 2 g of anhydrous sodium sulfate, 17 g of Florisil, 5 g of anhydrous sodium sulfate were examined intially. They were tapped gently to settle the contents and were washed with 20 ml of hexane which was allowed to pass through under gravity flow until dripping nearly stopped before the sample was added. Florisil at 2.4, 5.4 and 8.5% moisture was tested, as well as Florisil heated 24 h at 110° and brought to 3% moisture, and Florisil washed with benzene and hexane, heated 24 h at 110° and brought to 3% moisture. A mixture of Florisil–charcoal (5:1) was also examined. Suitable amounts of standards were applied directly to the columns in hexane. Columns with added standards were eluted with 100 ml of hexane, followed by intermediate polarity solvent mixtures of 20–90% benzene in hexane and finally with acetone–benzene (5:95) and the fractions were analyzed. Not all combinations were tried with all adsorbents. Preliminary results indicated that the Florisil with 8.5% moisture gave the best recoveries with benzene–hexane (80:20). The elution of standards from this adsorbent was examined further using 1–8% acetone in hexane and 10–50% chloroform in hexane as intermediate polarity solvents and acetone–hexane (10:90) and chloroform as final eluents. In a similar manner, silica gel,

TABLE II

RECOVERY OF INSECTICIDES FROM COLUMN CHROMATOGRAPHY

Results given as: upper 5 μg level/lower 0.5 μg level.

Adsorbent	*Column fraction*	*Solvent*	*Volume (ml)*	*Recovery (%)*			
				Permethrin	*Fenpropanate*	*Cypermethrin*	*Fenvalerate*
Florisil	2	Benzene-hexane (80:20)	100	98/100	94/87	94/96	96/91
	2	Acetone-hexane (2:98)	150	96/—	100/—	93/—	94/—
	2	Chloroform-hexane (50:50)	150	91/—	95/—	90/—	91/—
Silica gel	2	Benzene-hexane (80:20)	150	92/107	91/97	84/92	84/88
	2	Acetone-hexane (7.5:92.5)	150	79/—	81/—	81/—	79/—
	2	Chloroform-hexane (80:20)	150	98/—	95/—	92/—	96/—
Aluminum oxide	1	Hexane	100	103/100	0/0	0/0	0/0
	2	Benzene-hexane (80:20)	100	0/0	100/96	96/78	100/80
	1	Hexane	100	94/—	0/—	0/—	0/—
	2	Acetone-hexane (2:98)	150	0/—	90/—	78/—	87/—
	1	Hexane	100	not analyzed			
	2	Chloroform-hexane (50:50)	150	0/—	94/—	79/—	91/—
Activated alumina	2	Benzene-hexane (80:20)	100	95/—	24/—	26/—	5/—
	3	Acetone-benzene (5:95)	75	0/—	66/—	30/—	75/—

aluminum oxide and activated alumina were examined. The minimum polarity solvent mixtures that would elute the pyrethroids in volumes of 100–150 ml were determined in this way. The elution patterns and recovery data are summarized in Table II.

Crop extracts from untreated asparagus, carrots, tomatoes, tobacco, onions and radishes were initially chromatographed on Florisil and the benzene–hexane (80:20) eluates analyzed for interferences. A 10-ml portion of the desired extract (1 g/ml) was solvent exchanged with 3 × 15 ml of hexane to remove any traces of acetone before being applied to the column. Subsequently extracts of carrot, tomato and tobacco were examined using acetone–hexane (2:98) and chloroform–hexane (50:50) as eluting solvent. Extracts of onion and radishes were also examined on aluminum oxide and silica gel respectively using the solvent mixtures described in Table II.

RESULTS AND DISCUSSION

Hexane extraction of the approximately (1:3) acetone–water extracts of various plant material recovered the four pyrethroids in high yield. The acetone maceration of the crop was not as efficient in extracting the added pyrethroids from plant material. Re-extraction with acetone yielded additional amounts of the insecticides

(Table I). The overall efficiency and variability is in the range reported for many residue analyses. Recovery during the first extraction was not improved by simply washing the plant fiber in the Büchner funnel with additional acetone. No loss of insecticide was observed on subsequent storage of the fortified hexane extracts for several months over anhydrous sodium sulfate at freezer temperatures.

Elution of Florisil (8.5% water) with 100 ml portions of hexane or benzene–hexane mixtures of increasing polarity showed that at least 300 ml of hexane or 100 ml of benzene–hexane (10:90) could be passed through the column without eluting any of the pyrethroids. Permethrin began to elute in benzene–hexane (20:80) and was completely eluted with a subsequent fraction of benzene–hexane (40:60). Good recovery of all four insecticides (Table III) was obtained by elution of the Florisil with either benzene–hexane (80:20), acetone–hexane (2:98) or chloroform–hexane (50:50) following a first fraction of hexane. Fenpropanate was more strongly retained on the Florisil containing less moisture, *i.e.*, 2.4, 5.4% or 3% added after drying at 110° overnight, as it was not completely eluted with 100 ml of benzene–hexane (80:20) but was recovered with a more polar solvent. Only permethrin was recovered in high yield from the Florisil–charcoal column. Recovery of the other materials was 25–50% with benzene–hexane (80:20) and the remaining material could not be eluted with acetone.

The silica gel had elution characteristics similar to the Florisil with benzene–hexane (80:20) as solvent but increased proportions of acetone and chloroform were required in the hexane (Table II) to elute the materials completely with 150 ml of solvent. Recoveries were lower in the solvent containing acetone.

The pyrethroids had a different elution pattern on aluminium oxide. High recoveries of permethrin were obtained by elution with 100 ml of hexane. All the pyrethroids were mobile with hexane and the recoveries of fenpropanate and cypermethrin were 100 and 83%, respectively, with 400 ml of hexane. Fenvalerate was only poorly recovered in this 400 ml elution which was thought to be somewhat above the volume which could be used economically. Elution of the aluminum oxide with the same solvents as Florisil gave good recoveries of permethrin, fenpropanate and fenvalerate with the permethrin eluting in the hexane and the other two in the intermediate polarity solvents. In general, cypermethrin was not recovered as well from this adsorbent. The elution pattern on the activated alumina was very different from the aluminum oxide and resembled that of the silica type adsorbents. It was tested only at its natural moisture level of 1.5% and in this state only permethrin could be recovered in good yield with benzene–hexane (80:20) (see Table II). The recoveries of fenvalerate and cypermethrin remained fair and poor, respectively, even after elution with acetone–benzene (5:95). As crop cleanup could be achieved with the other adsorbents no further work was done on the alumina.

Florisil cleanup with benzene–hexane (80:20) elution following a 100 ml hexane fraction was sufficient for most of the crops. Tobacco still contained some later-eluting components which did not directly interfere with the analysis. Fresh onion extract contained two responses close to the retention time of fenpropanate on the OV-101 phase used for analysis. One was removed by Florisil and the other by aluminum oxide. Refrigerator-stored onions contained additional components which could be reduced to the equivalent of 0.05 ppm of fenpropanate by this procedure. Radish extract contained materials which eluted from the gas chromatograph as a broad tailing

solvent front rather than as distinct interfering peaks. Florisil chromatography did not reduce this front to an acceptable level. The materials producing the broad front were eluted from the aluminum oxide with hexane providing cleanup for fenpropanate, cypermethrin and fenvalerate but leaving permethrin with the interferences in the hexane fraction. Silica gel retained the radish interferences and allowed elution of all the materials in an benzene–hexane (80:20) fraction.

The 2% or 7.5% acetone in hexane fraction from the chromatography of the crop extracts on the three adsorbents contained a component which eluted from the gas chromatograph close to fenpropanate. It would interfere with analyses of low levels. These extracts were generally less free of minor interferences than the benzene–hexane (80:20) fractions. The 50% or 80% chloroform in hexane fractions contained higher concentrations of the interfering components. Fenpropanate analysis on this fraction was impossible in most cases and analysis of the other materials would definitely be questionable in some cases. The use of either of these solvent systems as described, for example, in an effort to reduce the use of benzene in a laboratory, will be at the expense of efficiency in the removal of interferences from plant extracts.

CONCLUSION

Effective procedures have been developed for the extraction of permethrin, fenpropanate, cypermethrin and fenvalerate from a variety of crops and the removal of crop extractives from these extracts by column chromatography to permit analysis of the pyrethroids at residue levels by GLC–ECD. These procedures represent our current "best" solution to a new analytical problem.

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