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DETERMINATION OF FLUORESCENT PESTICIDES AND METABOLITES BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatograph equipped with a C_8 reversed-phase column and fluorescence detector was investigated for the selective determination of toxic chemicals at residue (ng) levels. Of 75 compounds (pesticides, metabolites, and a few industrial chemicals) initially studied, 35 compounds were successfully chromatographed and detected at residue levels. An acetonitrile–water gradient mobile phase was used to chromatograph most of the naphthalene and heterocyclic compounds. Two hydroxycoumarin compounds were chromatographed with an acetonitrile–aqueous 1 % acetic acid mobile phase and detected after post-column addition of base for maximum fluorescence. The benzimidazole compounds were chromatographed using an acetonitrile–aqueous 0.07 *M* triethylamine–phosphate (pH 7.1) gradient mobile phase.

INTRODUCTION

Since the advent of organic pesticides in the 1930s, hundreds of these compounds have been developed for the control of pests. Most of the early pesticides were thermally stable and their residues on food products could be readily determined by gas–liquid chromatography (GLC). During the past 10 years, there has been a trend toward the use of pesticides which would degrade more readily and thus be less detrimental to the environment. Many of these newer pesticides are thermally labile and/or non-volatile and thus are not directly amenable to determination by GLC.

High-performance liquid chromatography (HPLC) has proven to be an excellent technique for determining many of these pesticides as evidenced in the review by Lawrence and Turton[1]. The UV detector is generally used to monitor the separated sample components in the column effluent. Most organic pesticides, metabolites, and industrial chemical residues which may be present in foods, as well as extracted food components, would be observed with the UV detector. This is especially evident when monitoring the HPLC column effluent at high detector sensitivity and at lower UV wavelengths.

Selective detection techniques are beginning to be used in HPLC for improving the qualitative aspect of the determination of organic compounds. One of these

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detection techniques, fluorescence, offers increased selectivity and in many cases increased sensitivity. A number of pesticides have been reported to fluoresce naturally[2–5]. Recently, several authors have used high-performance liquid chromatographs equipped with fluorescence detectors to selectively determine pesticide residues. Argauer[6] determined carbaryl and several other naphthalene pesticides by using a cyano-bonded column with a methylene chloride–methanol mobile phase. HPLC–fluorometric methods have also been reported for naphthaleneacetic acid[7] and thiabendazole[8] by using a reversed-phase column and isocratic aqueous mobile phases. Krause and August[9] determined seven naturally fluorescent pesticides and a synergist by using a C_8 bonded column and acetonitrile–water gradient mobile phase.

The purpose of the work reported here was to determine which pesticides could be chromatographed and detected at residue levels with the reversed-phase HPLC–fluorometric technique. Seventy-five compounds (pesticides, metabolites, and a few industrial chemicals) were selected for study based on their known fluorescence or chemical structures indicating possible fluorescence. Initially, studies were conducted to determine the fluorescence in neutral and basic media under static conditions. Those compounds found to fluoresce were selected for HPLC–fluorometric study.

EXPERIMENTAL

Reagents

Water was purified using a Milli-Q water purification system from Millipore (Bedford, MA, U.S.A.). Methanol and UV grade acetonitrile were distilled-in-glass quality from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Solvents for HPLC were vacuum degassed for 5 min before use. N,N-Dimethylformamide, phosphoric acid (85%), and triethylamine were HPLC grade from Fisher (Fair Lawn, NJ, U.S.A.). The sodium salt of hexanesulfonic acid, triethylamine (TEA) (initially used), tetrabutylammonium hydroxide, and tetrabutylammonium phosphate were from Eastman (Rochester, NY, U.S.A.). Tris(hydroxymethyl)aminomethane was from Chem Service (Media, PA, U.S.A.). Glacial acetic acid was reagent grade from J. T. Baker (Phillipsburg, NJ, U.S.A.). Ammonium acetate, diethylamine, sodium acetate, sodium hydroxide, disodium hydrogen phosphate, and sodium dihydrogen phosphate were reagent grade from Fisher. The aqueous HPLC buffers were prepared from degassed Milli-Q water and were at 0.05 *M* concentration unless stated otherwise. For the benzimidazole linearity studies, 10 ml of triethylamine was added to 985 ml of degassed Milli-Q water and mixed. This solution was then titrated with phosphoric acid (85%) to a pH of 7.1 $\pm$ 0.05, using a Model 701 digital pH meter from Orion Research (Cambridge, MA, U.S.A.).

The pesticides, metabolites, and industrial chemicals studied are listed in Table I. Aniline (>98%) was from Aldrich (Milwaukee, WI, U.S.A.) and 2-(3-butylureido)benzimidazole (BUB) (>99%) was provided by DuPont (Wilmington, DE, U.S.A.). All other compounds were reference standards obtained from the U.S. Environmental Protection Agency (Research Triangle Park, NC, U.S.A.). For the static fluorescence studies, stock solutions were prepared by dissolving 2.5 mg of standard in a 25-ml actinic volumetric flask with degassed acetonitrile. A dilution of this solution was made to give an acetonitrile–water (1:1) solvent mixture and standard concentration of 10 μg/ml. A second solution of the same standard concentra-

tion was prepared in a similar manner to give an acetonitrile–0.04 *N* aqueous sodium hydroxide (1:1) solution. For the HPLC studies, stock solutions of 2-aminobenzimidazole (2-AB), BH-584, BUB, methyl 2-benzimidazolecarbamate (MBC) and thiabendazole were prepared by dissolving 2.5 mg of each separately in 25-ml actinic volumetric flasks with 3 ml of dimethylformamide and diluting each to 25 ml with methanol. All other compounds were dissolved directly with methanol in actinic volumetric flasks. All subsequent dilutions were made with methanol.

Apparatus

A Perkin-Elmer (Norwalk, CT, U.S.A.) Model MPF-44A fluorescence spectrophotometer was used for the static fluorescence studies. Excitation and emission slits were maintained at 10 nm. Voltage was adjusted to 900 V at 210-nm excitation and the instrument was operated in the ratio mode.

A schematic diagram of the HPLC fluorometric system used in the study is shown in Fig. 1. The compound solutions were injected onto a reversed-phase HPLC analytical column equipped with a guard column using a Valco (Houston, TX, U.S.A.) Model 16 AS-7000 automatic sampler with a 10-μl injection loop. The guard column (7 cm × 2.1 mm I.D.) contained 25–37-μm Co-Pell ODS packing from Whatman (Clifton, NJ, U.S.A.). The analytical column (25 cm × 4.6 mm I.D.) contained 6-μm Zorbax C_8 spherical particles from DuPont. The columns were maintained at 35°C in a custom-built forced air oven. A 30-min linear gradient from 12 to 70% acetonitrile in water or aqueous buffer was used to elute the compounds of interest. To elute some compounds, the mobile phase was abruptly changed at 30 min to 100% acetonitrile for an additional 15 min. The gradient was formed and pumped through the column at 1.5 ml/min with an Altex (Berkeley, CA, U.S.A.) Model 322 MP programmable liquid chromatograph.

For coumafuryl and warfarin, 0.8 *N* sodium hydroxide solution was added to the column effluent at 0.5 ml/min through a Valco stainless-steel (ss) tee (0.74 mm I.D.) attached to the exit of the column. The resulting alkaline effluent flowed into a 3 m × 0.48 mm I.D. 321 ss tubing coil from Tubesales (Englewood, NJ, U.S.A.). This coil was maintained at ambient temperature for coumafuryl and warfarin. For the

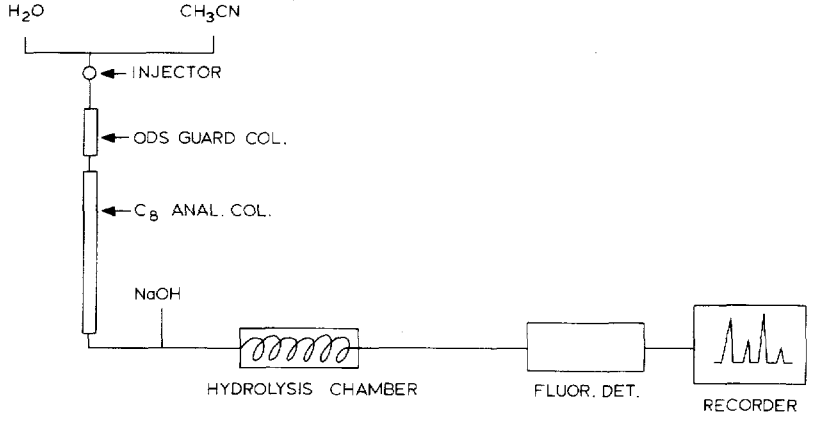


Fig. 1. HPLC–fluorometric system. Sodium hydroxide added only when required and hydrolysis chamber heated only when converting non-fluorescent compounds to fluorescent species.

benzotriazinone and pyrazinone compounds, 0.2 *N* sodium hydroxide solution was added to the column effluent at 0.5 ml/min. The hydrolysis coil was maintained at 100°C in an 18 × 18 × 13 cm column oven (obtained from a Model 5360 Barber-Colman gas chromatograph) controlled by a Model 700-115 proportional temperature controller from RFL Industries (Boonton, NJ, U.S.A.). By using a PTFE restriction coil (4 m × 0.5 mm I.D.) after the detector, a back pressure of 3.4 bar was applied to the hydrolysis coil to prevent boiling of the alkaline solution. The remaining compounds were passed through the same system, but no sodium hydroxide solution was added and the hydrolysis coil was maintained at ambient temperature.

The fluorescence of all compounds was monitored with a Perkin-Elmer Model 650-10LC detector equipped with a 20-μl cell. The excitation and emission wavelengths used are given in Table II. Excitation and emission slit widths were set at 15 and 12 nm, respectively. A 1-sec time constant was used. Sensitivity of the detector was adjusted so that with the acetonitrile–water gradient 50 ng of carbofuran produced 50% full-scale response on a Spectra-Physics (Santa Clara, CA, U.S.A.) Model 4000 microprocessor-printer plotter (detector PM gain, low; sensitivity range, 30; and printer plotter attenuation, 5). Baseline noise should be 1% or less.

The sodium hydroxide solution was added to the column effluent with an Oyster Bay (Oyster Bay, NY, U.S.A.) Model MCH-0-100 pump. Pulsations were dampened with a 40 cm × 0.48 mm I.D. ss tubing air dampener before passing the solution through 1 m of 0.018 mm I.D. No. 304 ss tubing and then into the column effluent through the ss tee. No. 304 ss tubing (1.6 mm O.D. × 0.018 mm I.D.) was used to connect injector, columns, and tee. A 20-cm length of the same tubing was used to connect hydrolysis coil to detector tubing and acted as heat sink to reduce solution temperature to near ambient.

RESULTS AND DISCUSSION

Fluorescence

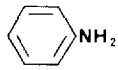
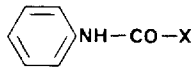
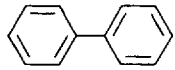
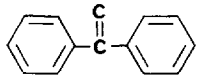
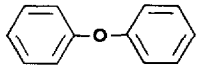
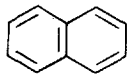
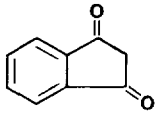
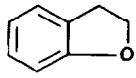
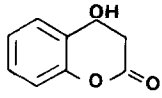
The relative fluorescent intensity of the 75 pesticides/metabolites and industrial chemicals obtained in neutral and basic media under static conditions is given in Table I. An acetonitrile–water (1:1) solution was the neutral medium for the compounds, and was selected based on its anticipated use as an HPLC mobile phase. To study the effect of a basic medium on the compounds, an acetonitrile–aqueous 0.04 *N* sodium hydroxide (1:1) solution was used.

Aniline, an herbicide metabolite and industrial chemical, and biphenyl, a fungicide, exhibit intense fluorescence. However, with halogen substituents on the ring, fluorescence is reduced or eliminated. This general phenomenon has been discussed by Wehry[10]. The naphthalene pesticides that contain amide, carboxylic acid, ester, and phenolic substituents generally exhibited intense fluorescence.

Many of the compounds studied had heterocyclic structures. Those compounds having the benzimidazole, benzodihydroxyfuran, benzodihydroxypyran, benzothiopene, coumafuryl, or quinoline structures generally exhibited moderate to intense fluorescence. An exception is the non-fluorescent 4-hydroxy metabolite of fenazaflor (5,6-dichloro-1-phenoxycarbinyl-2-trifluoromethylbenzimidazole). Closely related to benzimidazole is the benzothiazine structure in which a ring nitrogen atom is replaced with a sulfur atom. Although the benzimidazole compounds generally

TABLE I

LIST OF 75 COMPOUNDS AND THEIR RELATIVE FLUORESCENCE IN NEUTRAL AND BASIC SOLUTION

General structure		*Compound*	*Rel. fluorescence**	
			Neutral media	*Basic media*
Aniline		Aniline	H	H
		p-Chloroaniline	M	M
		3,4-Dichloroaniline	O	O
Anilide		Propham	M	M
		Fenuron	L	L
		Carboxin	O	O
Biphenyl		Biphenyl	H	H
		o-Phenylphenol	H	H
		Polychlorinated biphenyl (Aroclor 1242)	L	L
		Polybrominated biphenyl	O	O
Bisphenylethene		Perthane olefin	O	O
Diphenyl ether		Permethrin	L	L
Naphthalene		Carbaryl	H	H
		Naphthalene acetamide	H	H
		Naphthaleneacetic acid	H	H
		Naphthaleneacetic acid methyl ester	H	H
		Napropamide	H	H
		α-Naphthol	M	H
		Naphthoxyacetic acid	M	H
		Naptalam	M	L
		Naptalam, sodium salt	L	L
Indandione		Pindone	O	O
Benzodihydrofuran		Ethofumesate	H	H
		Carbofuran	H	L
		3-Hydroxycarbofuran	H	L
Hydroxycoumarin		Coumafuryl	M	M
		Warfarin	M	M

(Continued on p. 502)

TABLE I *(continued)*

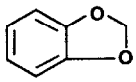
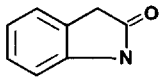
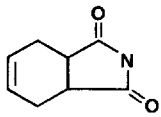
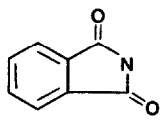
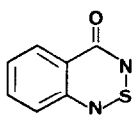
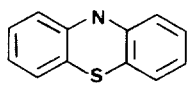
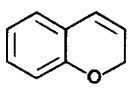
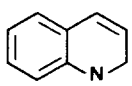
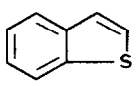
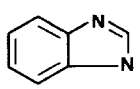
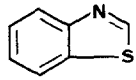
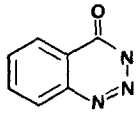
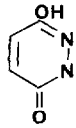
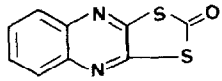
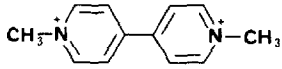
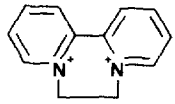
General structure		*Compound*	*Rel. fluorescence**	
			Neutral media	*Basic media*
Benzodioxy		Piperonyl butoxide	H	H
		Piperonyl cyclohene	M	M
		Bendiocarb	O	O
Benzoxolin		Phosalone	M	M
		Phosalone oxygen analogue	M	M
Dicarboximide		Captofol	O	O
		Captan	O	O
		MGK 264	O	O
Phthalimide		Folpet	O	L
		Dialifor	O	O
		Emmi	O	O
		Phosmet	O	O
		Phosmet oxygen analogue	O	O
			O	O
		Tetramethrin	O	O
Benzothiadiazinone		Bentazon	M	M
		6-Hydroxybentazon	L	O
		8-Hydroxybentazon	L	O
Phenothiazine		Phenothiazine	L	L
Benzodihydropyran		Coumaphos	H	O
		Coumaphos oxygen analogue	H	O
Quinoline		Ethoxyquin	H	H
Benzothiophene		4-Hydroxybenzothiophene	M	M
		Mobam	M	M
Benzimidazole		2-AB	H	H
		BH-584	H	H
		BUB	H	H
		Thiabendazole	H	H

TABLE I *(continued)*

General structure	*Compound*	*Rel. fluorescence**	
		Neutral media	*Basic media*
	Benomyl	M	M
	MBC	M	M
	Fenazaflor	M	L
	Fenazaflor A	M	L
	Fenazaflor B	O	O
Benzothiazole 	MBT	O	O
	TCMTB	O	O
Benzotriazinone 	Azinphos ethyl	O	M**
	Azinphos methyl	O	M**
	Azinphos methyl oxygen analogue	O	M**
Pyridazinone 	Maleic hydrazide	L	O
Quinomethionate 	Quinomethionate	M	O
Pyrazinone 	Thionazin	O	M**
	Thionazin oxygen analogue	O	M**
Bipyridinium compounds 	Paraquat dichloride	O	O
	Paraquat bis (methylsulfate)	O	O
Bipyridylium compounds 	Diquat dibromide	L	L
	Diquat bis(tribromide)	L	O
Complex	Cerasan M	L	L
	Norbormide	L	L
	Rotenone	O	L

* Neutral media: acetonitrile–water (1:1). Base media: acetonitrile–aqueous 0.4 *N* sodium hydroxide (1:1). H = High fluorescence (instrument sensitivity range 0.1–0.3); M = medium fluorescence (instrument sensitivity range 1–3); L = low fluorescence (instrument sensitivity range 10); O = no fluorescence (instrument sensitivity range 10). Least sensitivity setting = 0.1; high sensitivity setting = 10.

** Fluorescence after heating solution.

fluoresce, the benzothiazine compounds studied in this work were non-fluorescent. The bisphenylethene and dipyridinium compounds were also non-fluorescent. Those compounds having the dicarboximide, indandione, phthalimide, and pyridazine structures were non-fluorescent except for low fluorescence of folpet in basic medium and maleic hydrazide in neutral solution.

Some compounds do not fluoresce naturally, but hydrolyze to fluorescent derivatives. The non-fluorescent benzotriazinone compounds (azinphos ethyl, azinphos methyl, and azinphos methyl oxygen analog) can be hydrolyzed with base to fluorescent anthranilic acid as reported by MacDougall[3]. Likewise, the pyrazine compounds do not fluoresce until hydrolyzed by base to sodium pyrazinolate as reported by Kiigemagi and Terriere[11]. Rotenone, which was non-fluorescent in the neutral medium, also appeared to degrade to a fluorescent derivative in the basic medium as fluorescent response increased with time.

The fluorescent intensity of some of the compounds was affected by the pH of the solution. The benzodihydrofuran and benzodihydropyran compounds had intense fluorescence in the neutral medium, but exhibited low fluorescence or non-fluorescence in the basic medium. This phenomenon was also observed for other compounds, but to a lesser degree. Although aniline exhibited intense fluorescence in the neutral and basic media, Bridges and Williams[12] reported that aniline does not fluoresce in acidic media. In the HPLC studies reported here, the fluorescent intensity of coumafuryl and warfarin also decreased in an acidic mobile phase.

Of the 75 compounds studied, 55 exhibited low to intense fluorescence, either naturally or through hydrolysis to fluorescent derivatives. Generally, fluorescence of the compounds appears related to molecular structure and substituents, and can be affected by the pH of the solution.

HPLC–fluorescence

Forty-six of the 55 pesticides, metabolites, and industrial chemicals found to fluoresce naturally, or to hydrolyze to fluorescent derivatives, were investigated for determination by a high-performance liquid chromatograph equipped with a fluorescence detector. Table II lists the 43 compounds which were found to chromatograph satisfactorily and gives information on the compounds' excitation and emission wavelengths, chromatographic retention, and fluorescence intensity. Compound retention is relative to carbofuran, whose retention was always obtained using the acetonitrile–water gradient mobile phase. The linear response range was determined for those pesticides that are commercially produced and their metabolites whose fluorescent intensity was equal to or more than 50% full-scale deflection (f.s.d.) for 100 ng.

Twenty-five naturally fluorescent compounds produced sharp symmetrical chromatographic peaks using the acetonitrile–water gradient mobile phase. A chromatogram of several of these compounds is shown in Fig. 2. Aniline and 6-hydroxybentazon produced symmetrical but slightly broader peaks with the same mobile phase. For twelve compounds 10 ng or less produced peaks of 50% f.s.d. The linear response range was somewhat limited at higher concentrations due to the reabsorption of fluorescent energy by unexcited compounds. Unlike static fluorescence, where total quenching can occur, a fluorescent peak or doublet will always be observed when this occurs. Fig. 3 shows linearity curves for naphthalene acetamide (NAD) and

TABLE II

HPLC–FLUORESCENCE CHARACTERISTICS OF 43 COMPOUNDS

Compound	*Maximum wavelength (nm)*		*Wavelengths (nm) used with HPLC*		*Retention time relative to carbofuran***	*Amount producing 50% f.s.d.**** *response (ng)*	*Linear response range (ng)*
	E_x*	E_m*	E_x	E_m			
Acetonitrile–water gradient mobile phase							
Aniline	245	340	245	340	0.82	40	0.5–125
Biphenyl	258	316	258	316	1.55	3	0.2–10
Carbaryl	286	338	288	330	1.06	3	0.5–10
Carbofuran	278	306	288	330	1.00	90	10–250
p-Chloroaniline	292	343	292	343	0.99	200	
Coumaphos	320	380	320	385	1.61	5	0.5–14
Coumaphos oxygen analogue	320	388	320	385	1.22	4	0.5–12
Ethofumesate	285	320	288	330	1.42	60	2–200
Ethoxyquin	358	446	358	446	1.73	250	
Fenazaflor	270	358	270	358	1.72	50	
Fenazaflor A	276	355	276	355	1.67	30	
6-Hydroxybentazon	366	475	366	475	0.17	250	
4-Hydroxybenzothiophene	264	335	264	335	1.03	100	
3-Hydroxycarbofuran	284	310	288	330	0.60	30	2–100
Mobam	266	320	266	320	1.01	80	
Naphthalene acetamide	286	335	288	320	0.75	3	0.5–9
Naphthaleneacetic acid methyl ester	286	338	288	330	1.44	4	0.5 10
α-Naphthol	308	460	308	460	1.10	6	1–20
Napropamide	296	342	288	330	1.36	4	0.5–12
Naptalam	328	427	328	427	1.13	6	
o-Phenylphenol	287	336	288	330	1.25	4	0.5–12
Phosalone	287	320	288	330	1.70	90	10–250
Phosalone oxygen analogue	288	318	288	330	1.30	90	8–250
Piperonyl butoxide	295	330	288	330	1.74	5	0.5–12
Piperonyl cyclonene	268	345	268	345	1.41	250	
Propham	242	306	242	306	1.81	250	
Quinomethionate	362	395	362	395	1.51	4	0.5–13
Acetonitrile–water gradient mobile phase and post-column basic hydrolysis							
Azinphos ethyl	320	400	320	400	1.46	5	0.5–18
Azinphos methyl	320	400	320	400	1.29	5	0.5–16
Azinphos methyl oxygen analogue	320	400	320	400	0.81	15	0.5–40
Rotenone	255	403	255	403	1.44	1000	
Thionazin	324	385	324	385	1.23	180	
Thionazin oxygen analogue	324	385	324	385	0.70	50	
Acetonitrile–1% acetic acid in water gradient mobile phase							
Naphthaleneacetic acid	289	333	288	330	1.00	5	0.5–15
Naphthoxyacetic acid	276	355	276	335	0.99	3	0.5–8

(Continued on p. 506)

TABLE II *(continued)*

Compound	*Maximum wavelength (nm)*		*Wavelengths (nm) used with HPLC*		*Retention time relative to carbofuran***	*Amount producing 50% f.s.d.**** *response (ng)*	*Linear response range (ng)*
	E_x*	E_m*	E_x	E_m			
Acetonitrile–1% acetic acid in water gradient mobile phase and post-column addition of base							
Coumafuryl	315	394	315	395	1.13	20	2–40
Warfarin	317	390	315	395	1.25	11	1–35
Acetonitrile–0.072 M triethylamine in water adjusted to pH 7.1 with H_3PO_4 gradient mobile phase							
2-AB	286	322	286	322	0.40	10	1–30
BH-584	280	305	280	305	0.99	10	
BUB	286	310	286	322	1.03	3	0.5–8
MBC	286	310	286	322	0.63	35	2–100
Phenothiazine	255	450	255	450	1.45	100	10–300
Thiabendazole	305	357	305	357	0.75	4	0.5–10

* E_x = excitation, E_m = emission.

** Carbofuran chromatographed using acetonitrile–water gradient mobile phase: retention time approximately 20 min.

*** f.s.d. = Full-scale deflection.

azinphosmethyl, which are similar to those obtained for other compounds.

The three benzotriazinone compounds (azinphos ethyl, azinphos methyl, and azinphos methyl oxygen analogue), two pyrazine compounds (thionazin and thionazin oxygen analogue) and rotenone chromatograph well with the acetonitrile–water gradient mobile phase. The benzotriazinone and pyrazine compounds have similar excitation and emission wavelengths but are adequately separated with the liquid chromatograph (Table II). However, post-column addition of aqueous sodium hydroxide solution and heat is required to produce fluorescence. The effect of sodium hydroxide concentration (post-column addition at 0.5 ml/min) with the hydrolysis chamber maintained at 100°C for the benzotriazinone and pyrazine compounds is shown in Fig. 4. For the benzotriazinone compounds, which are commercially important pesticides, the 0.2 *N* sodium hydroxide solution appeared near optimum, whereas for the pyrazine compounds, a base concentration near 0.8 *N* appeared near optimum. For the benzotriazinone compounds, excess base concentration reduced fluorescence. Further study will be required to understand this phenomenon. The 0.2 *N* sodium hydroxide solution was used to obtain response data for these five compounds because only the benzotriazinone (azinphos) pesticides are currently of commercial significance.

Coumafuryl, naphthaleneacetic acid, naphthoxyacetic acid, and warfarin did not chromatograph well with the acetonitrile–water mobile phase. Addition of glacial acetic acid (1%) to the water improved peak shape and provided reproducible retention times (Table II) for the two organic acids. However, the acidic medium reduced the fluorescence of coumafuryl and warfarin. Post-column addition of 0.8 *N* sodium hydroxide at 0.5 ml/min provided the basic environment required for maximum fluorescence of coumafuryl and warfarin.

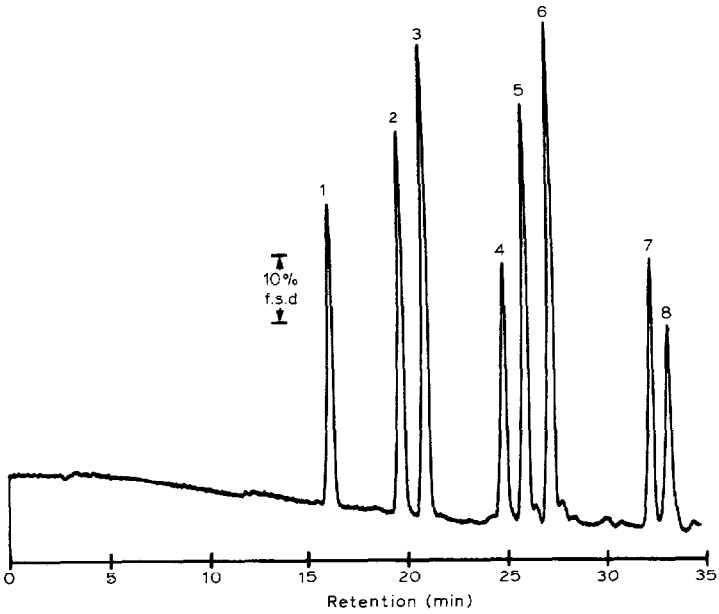


Fig. 2. HPLC chromatogram of seven pesticides and a synergist that fluoresce naturally. Peaks: 1 = NAD (3 ng); 2 = carbofuran (120 ng); 3 = carbaryl (6 ng); 4 = phosalone oxygen analog (80 ng); 5 = napropamide (7 ng); 6 = naphthaleneacetic acid methyl ester (8 ng); 7 = phosalone (90 ng); 8 = piperonyl butoxide (3 ng). E_x = 288 nm, E_m = 330 nm.

The benzimidazole compounds were chromatographically the most challenging. Benomyl produced several broad peaks with the acetonitrile–water gradient mobile phase. Its rapid degradation in organic solvents has been well documented[13,14] and thus direct determination of benomyl by HPLC is not feasible. MBC produces a rather broad peak with the above mobile phase. 2-AB, BH-284, BUB, phenothiazine, and thiabendazole did not chromatograph with the acetonitrile–water mobile phase. With an acetonitrile–aqueous 1% acetic acid mobile phase, the compounds did elute, but as broad tailing peaks. Peak tailing of the compounds was also observed when ammonium acetate, diethylamine (to pH 7 with acetic acid), hexanesulfonic acid (as sodium salt), sodium phosphate (pH 7), or tris(hydroxymethyl)-aminomethane (to pH 7 with phosphoric acid) was added to the aqueous portion of the mobile phase at 0.05 *M* concentration. In addition, the organic buffers contained extraneous materials which were observed with the fluorescence detector. The chromatography of the benzimidazole compounds was best with either TEA or tetrabutylammonium hydroxide aqueous buffers adjusted to pH 7. TEA was the buffer of choice because it is reasonably free of extraneous peaks (see Fig. 5) and the HPLC grade of TEA costs less than reagent grade tetrabutylammonium hydroxide. Adjustment of the aqueous TEA mobile phase to pH 7.1 produced near optimum peak retention (separation and stability) and maximum response for 2-AB, BUB, and MBC. Also, at this pH HPLC column stability was not sacrificed. Adjustment of pH near 7 was most easily attained using phosphoric acid, which has a pK_a at 7.2, and thus was used rather than acetic acid, which has a pK_a at 4.8. The effect of TEA concentration on chromatography of 2-AB, BUB, and MBCwas studied. At a 0.02 *M* TEA concentra-

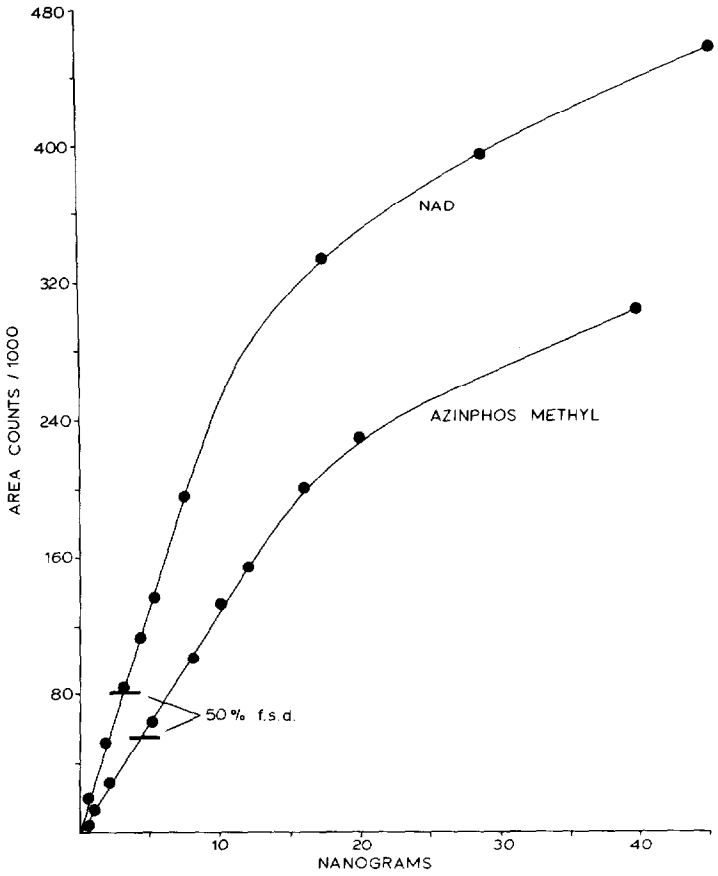


Fig. 3. Representative HPLC–fluorometric linearity curves.

tion 2-AB and MBC coeluted; however, all three peaks were separated at the 0.05 and 0.1 *M* TEA concentrations and the BUB peak was slightly sharper. For convenience, a 0.072 *M* TEA solution was used (10 ml TEA per l of solution). The effect of column reequilibration time on stability of peak retention and response was investigated. Reequilibration times from 10 to 60 min did not affect the stability of peak retention or response. A 15-min reequilibration time was selected.

The chromatography achieved for 2-AB, BUB, and MBC using the established parameters is shown in Fig. 5. The retention and response data obtained for the six benzimidazole compounds are presented in Table II. All except BH-584 and BUB were adequately separated. Except for MBC and phenothiazine, 10 ng or less of the benzimidazole compounds produced a peak of 50% FSD. The energy output of the xenon lamp used is fairly low at the 255 nm wavelength used for phenothiazine. Its response could possibly be improved by using a fluorescence detector with a deuterium lamp such as the Kratos Model 970. Diquat dibromide and diquat bis(tribromide) did not chromatograph satisfactorily with any of the mobile phases investigated using the octylsilane (C_8) column. Bentazon, like benomyl, was found to

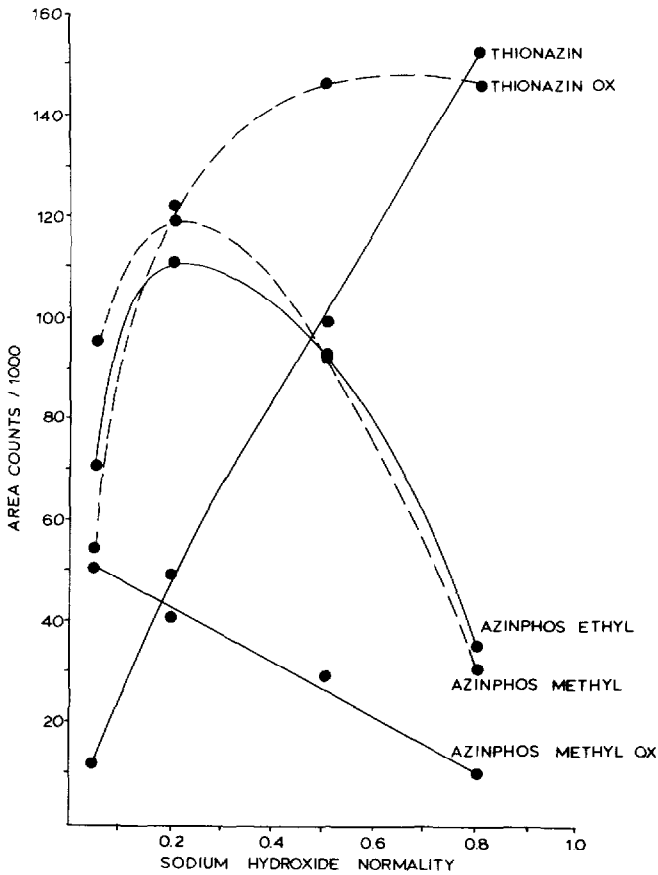


Fig. 4. Effect of sodium hydroxide concentration on response of benzimidazole and pyrazinone pesticides to fluorometric detector.

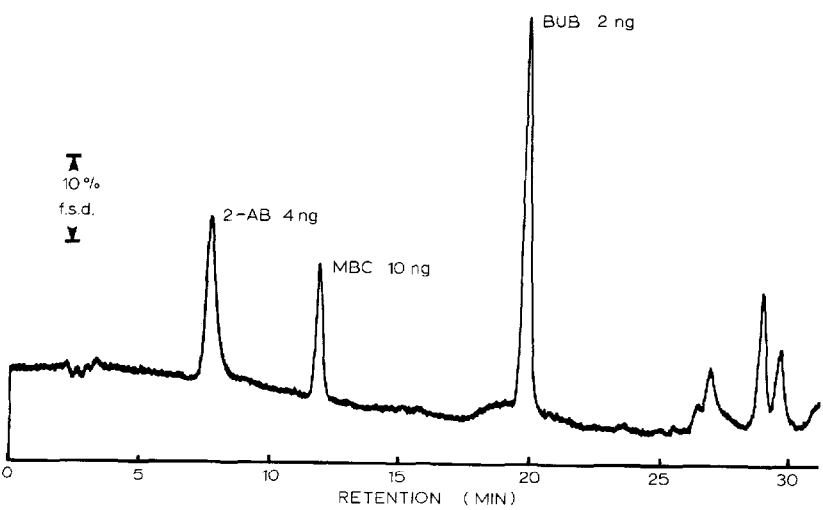


Fig. 5. HPLC chromatogram of three benzimidazole compounds. E_x = 286 nm, E_m = 320 nm.

rapidly degrade in solution; therefore, its direct determination by HPLC is not feasible.

The HPLC–fluorometric technique has recently been used in conjunction with a carbamate insecticide multiresidue method[15] for the determination of several fluorescent pesticides using the acetonitrile–water gradient mobile phase[9]. Information on pesticide recovery and effect of detector excitation and emission wavelengths on selectivity of pesticide response relative to crop coextractives is reported.

CONCLUSION

Thirty-five pesticides and metabolites were satisfactorily chromatographed and detected at residue levels (100 ng or less for 1/2 f.s.d.) with the HPLC–fluorometric determinative system. Most of the compounds were individually determined without interference from other compounds by a combination of chromatographic separation and fluorometric selectivity through the use of different excitation and emission wavelengths. Thus, the HPLC–fluorometric system enables the individual determination at residue levels of most of the pesticides and metabolites which naturally fluoresce or hydrolyze to fluorescent compounds.

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