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Note

Application of high-performance liquid chromatography to the determination of pesticides included in the European Economic Community Directive on fruit and vegetables*

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In November, 1976, the European Economic Community (EEC) adopted a Directive¹ which sets the strictest permissible maximum levels for residues of certain pesticides in or on fruit and vegetables where member countries decide to adopt such limits. Of the forty-two pesticides listed, eight are organochlorine compounds, nineteen are organophosphorus compounds and the remainder include a mixture of carbamates, dinitro compounds, triazines, trichloromethyl-thio compounds, substituted phenoxy acids, substituted phenylureas, etc. (Table I). There are no simple multi-residue screening procedures which will detect such a varied combination of pesticide classes to check whether or not a sample conforms with the Directive, although there are a number of multi-residue methods available for the determination of organochlorine and organophosphorus pesticides using gas-liquid chromatography (GLC). Very few multi-residue procedures using liquid chromatography have been published. Lawrence^{2,3} has developed methods for the determination of carbamates and substituted phenylurea herbicides in foodstuffs. Farrow *et al.*⁴ have reported a procedure for the determination of post-harvest fungicides on citrus fruit, and a method for the examination of grain, soil and river water for substituted phenylurea herbicides has been published by Farrington *et al.*⁵.

An investigation has been carried out to assess the usefulness of reversed-phase liquid chromatography using UV detection and gradient elution for the determination of residues of those pesticides listed in the EEC Directive. It has been shown that most of the compounds can be separated and detected by this system, although the sensitivity achievable with the UV detector is unlikely to be sufficient to detect some of these compounds at or near the EEC limit.

MATERIALS AND METHODS

Acetonitrile is, ideally, HPLC far UV grade. For routine purposes, acetonitrile, HPLC grade S, from Rathburn Chemicals (Walkerburn, Great Britain), is suitable.

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TABLE I

UV CHARACTERISTICS OF THE PESTICIDES LISTED IN THE EEC DIRECTIVE

A Pye-Unicam SP1800 spectrophotometer was utilized for all measurements using spectrograde methanol as solvent. Molar extinction coefficients (E) are $\times 10^3$.

Pesticide	EEC limit (ml/kg)	λ_{max}	E_{max}	λ_1^{**}	λ_1^{**}	(E_1)	λ_2	(E_2)	λ_3	(E_3)	E_{210}	E_{230}	E_{354}	E_{280}	E_{245}
Amitrole	nil	207	5.0	—	—	—	—	—	—	—	4.6	2.1	<0.05	<0.05	0.08
Aramite	nil	sample not available													
Atrazine	1.0	224	38.8	265	3.3	—	—	—	—	—	14.0	36.2	2.9	1.2	4.9
Azinphos-ethyl } Azinphos-methyl }	root veg. nil, other 0.4	225	28.3	287	7.8	—	—	—	—	—	21.7	27.5	5.9	7.4	8.6
		223	31.7	285	8.2	—	—	—	—	—	25.4	31.4	6.1	7.9	9.2
Barban	0.1	209	38.7	240	16.6	280	1.2	—	—	—	38.2	8.5	2.6	1.2	14.2
Binapacryl	carrots nil, other 0.3	230	23.7	—	—	—	—	—	—	—	20.6	22.2	20.0	8.7	22.5
Captan	15.0	208	6.8	—	—	—	—	—	—	—	6.3	2.4	0.3	<0.05	0.3
Carbaryl	some fruit, veg. 2.5, other 1.2	219	90.0	282	6.6	272	5.7	—	—	—	41.8	86.8	2.2	6.4	1.2
Chlorbenside***	1.5	<205	20.2	220	19.9	258	10.8	—	—	—	18.2	19.9	9.9	3.4	8.1
Chlorfenson***	1.5	<205	19.4	230	18.3	—	—	—	—	—	13.3	15.6	1.6	0.5	3.0
Chlorbenzilate***	1.5	<205	22.9	227	21.4	—	—	—	—	—	21.8	18.9	0.6	0.2	2.3
Chloroxuron	0.2	250	24.4	210	24.3	—	—	—	—	—	24.3	12.8	21.8	3.0	23.5
Demeton-S-methyl	carrots nil, other 0.4	210	3.6	—	—	—	—	—	—	—	3.6	1.7	<0.05	<0.05	0.5
Demeton-S-methyl***		<205	0.8	—	—	—	—	—	—	—	0.8	0.7	<0.05	<0.05	<0.05
Demeton-S-sulphone															
Diallate	0.05	210	16.6	—	—	—	—	—	—	—	16.6	11.3	0.3	<0.05	0.9
Dichlorprop***	0.05	<205	18.0	230	8.34	285	2.1	—	—	—	14.1	7.3	0.4	1.7	0.8
Dimethoate***	1.5	<205	8.1	—	—	—	—	—	—	—	6.8	3.4	0.2	<0.05	0.6

NOTES

Dinoseb	0.05	215	13.9	266	13.8	—	13.2	11.4	11.5	10.8	8.9
Dodine***	fruits 1.0, others nil	<205	4.6	—	—	—	1.1	<0.05	<0.05	<0.05	<0.05
Endosulfan	carrots 0.2, other 0.5	216	7.5	—	—	—	6.9	6.5	0.3	<0.05	0.7
Endrin	nil	225	3.8	—	—	—	3.1	3.4	0.2	<0.05	1.3
Fenchlorphos	0.5	212	18.4	230	8.9	283	1.2	291	1.1	0.9	1.0
Fenitrothion	0.5	208	14.5	268	7.1	—	14.1	8.2	5.3	6.1	3.7
Folpet	15.0	222	48.6	—	—	—	29.6	47.4	2.0	1.5	3.0
Formothion***	0.1	<205	8.5	221	5.4	—	6.9	5.4	0.6	0.5	0.7
γ -HCH***	leaf veg. 2.0, carrots 0.1, other 1.5	<205	0.8	—	—	—	0.5	<0.05	<0.05	<0.05	<0.05
Malathion***	veg. except root veg. 3.0, other 0.5	<205	6.3	—	—	—	5.6	2.6	0.8	<0.05	0.5
Malaoxon	10.0	sample not available	—	—	—	—	—	—	—	—	—
Methoxychlor***	0.4	<205	25.6	227	17.4	243	13.8	273	3.5	16.6	13.6
Omethoate***	carrots nil, other 0.4	<205	5.3	—	—	—	3.8	1.4	0.2	<0.05	0.3
Oxydemeton-methyl	0.5	<205	3.1	—	—	—	2.3	1.5	<0.05	<0.05	<0.05
Parathion***	0.15	<205	13.9	274	10.3	—	12.2	8.7	6.4	9.9	4.4
Paraoxon***	0.15	<205	11.6	216	9.1	271	9.9	8.8	8.3	9.1	4.4
Paraoxon-methyl}	0.15	<205	13.9	273	10.7	—	11.8	8.9	6.8	10.0	4.6
Phosphamidon	0.15	sample not available	—	—	—	—	—	—	—	—	—
Propoxur***	3.0	<205	10.0	—	—	—	9.6	7.9	1.0	0.15	1.9
TEPP	nil	<205	11.5	221	7.2	274	1.8	6.8	7.1	1.5	0.1
Thiram	strawberries 3.8, other 3.0	<205	0.7	—	—	—	0.6	0.4	<0.05	<0.05	0.1
Camphechlor	0.4	218	21.4	280	11.8	—	19.1	21.1	13.0	11.8	13.6
Trichlorphon***	0.5	<205	1.6	—	—	—	1.6	1.4	<0.05	<0.05	0.3
		<205	0.7	—	—	—	0.5	0.1	<0.05	<0.05	<0.05

* Wavelength of maximum absorption (nm).

** λ_1 - λ_3 are wavelength maxima of additional UV bands in order of decreasing intensity.

*** The λ_{max} for these compounds lies below 205 nm. The extinction coefficient was calculated for 205 nm.

Water, double distilled, is used. Standard solutions of the pesticides were prepared in methanol at concentrations of 100 $\mu\text{g}/\text{ml}$ and diluted as necessary. The UV spectra of the pesticide solutions were recorded on a Pye-Unicam SP1800 spectrophotometer.

Liquid chromatography

Two Waters Assoc. constant-volume solvent delivery systems, Model 6000A, and a solvent flow programmer, Model 660, were used. A variable-wavelength ultra-violet detector (Cecil Instruments, Model CE 212) fitted with a 10- μl flow cell was employed. A stainless-steel column, 150 \times 4.6 mm I.D. was slurry packed with 5- μm ODS-bonded silica (Spherisorb ODS) in methanol at 5000 lb./in.². A fine-mesh wire disc was fitted on top of the column packing material and this was covered with a 5-mm layer of 80-mesh silanised glass beads and finally a PTFE plug. An aliquot of the pesticide solution was injected via a needle guide onto the centre of the disc using a stop-flow technique.

The column was eluted with various mixtures of acetonitrile-water to establish the most suitable conditions for the various pesticides at a flow-rate of 1 ml/min. Finally a gradient elution from 25% to 75% acetonitrile in water was used.

RESULTS AND DISCUSSION

The UV absorbance characteristics of the various pesticides are given in Table I. From these data it is apparent that certain pesticides on the list do not absorb sufficiently in the UV region to enable the possible determination of residues using UV detection. Other pesticides possess strong absorption only below wavelengths of about 210 nm ("end absorbance") which could produce difficulties when screening extracts, because of absorption of non-pesticide components at short wavelengths. A "suitability factor" has been calculated (Table II) which gives the relative usefulness of UV detection for determining the pesticides at the EEC limit when monitoring at the absorption maximum.

$$\text{Suitability factor} = \frac{\text{EEC limit for pesticide} \times E_{\text{max. for pesticide}} \times 1000}{\text{EEC limit for folpet} \times E_{\text{max. for folpet}}}$$

Folpet was chosen as the standard for comparison because the product of the EEC limit and the extinction coefficient was the highest for any of the pesticides in the EEC Directive. For pesticides exhibiting only "end absorbance" the "suitability factor" was calculated using the extinction coefficient at 205 nm. It is unlikely that pesticides with "suitability factors" less than five could be determined with sufficient sensitivity by high-performance liquid chromatography (HPLC) using UV detection. Based on the results from Table II the pesticides to which this system can most usefully be applied, apart from the organochlorine and organophosphorus pesticides, are folpet, carbaryl, captan, thiram, atrazine, propoxur, and possibly binapacryl, chloroxuron, dodine and barban depending on the efficiency of the clean-up. For multi-residue detection a single wavelength would normally be chosen for monitoring. This will depend on the pesticides to be determined and the purity of the solvents available. In this work 215 nm proved to be a convenient wavelength. "Suitability factors" are shown in Table II calculated from the extinction coefficients of the pesticides at 215 nm and the extinction coefficient for folpet at $\lambda_{\text{max.}}$.

TABLE II

RELATIVE SUITABILITY OF UV DETECTION FOR DETERMINING PESTICIDES AT THE EEC LIMIT AT λ_{max} . AND 215 nm

Pesticide	Suitability factor	
	at λ_{max} .	at 215 nm
Folpet	1,000	740
Methoxychlor	350	170
Carbaryl	310 or 150	250 or 120
Captan	140	100
Thiram	113 or 88	87
Atrazine	53	36
Propoxur	47	35
Chlorobenzilate	47	39
Chlorbenside	42	39
Chlorfenson	40	27
Malathion	26 or 4.3	22 or 4
Azinphos-methyl	17 or 2.2	16 or 2
Dimethoate	17	11
Azinphos-ethyl	15 or 1.9*	13 or 1.6*
Fenclorphos	13	12
Fenitrothion	10	8
Binapacryl	10 or 1.6*	9 or 1.5*
Parathion	9.5	7
Paraoxon	8	6.5
Chloroxuron	6.7	5.0
Dodine	6.3 or 0.03*	0.6 or 0.003*
Barban	5.3	3.5
Endosulfan	5.1 or 2.0	5.0 or 2.0
Omethoate	2.9	1.2
Parathion-methyl	2.9	2.2
γ -HCH	2.2 or 1.6 or 0.1	0.7 or 0.5 or 0.03
Phosphamidon	2.0	1.8
Demeton-S-methyl	2.0 or 0.25*	1.6 or 0.2*
Oxydemeton-methyl	1.7 or 0.21*	1.0 or 0.13*
Dichlorprop	1.2	0.6
Formothion	1.2	0.9
Diallate	1.1	1.0
Dinoseb	0.75	0.95
Camphechlor	0.88	0.88
Trichlorphon	0.48	0.16
Demeton-S-methyl sulphone	0.44 or 0.05	0.44 or 0.05
Amitrole*	0.34	0.27
Endrin*	0.26	0.19
TEPP*	0.05	0.04

* Zero tolerance taken as 0.05 mg/kg.

Reversed-phase HPLC using an ODS-bonded silica column and acetonitrile-water mixtures as mobile phase (methanol-water having been found less suitable) was considered likely to give the most efficient separation of the pesticides. Sparacino and Hines⁶ found this system useful for the separation of carbamate pesticides and other workers^{7,8} have found reversed-phase chromatography useful for the separation of pesticides.

The relative elution order of the pesticides with sufficient absorbance in the UV region is listed in Table III and was obtained by injecting individual pesticides at several different acetonitrile-water concentrations ranging from 25% to 75% acetonitrile in water. Pesticides bracketed together could not be separated under isocratic conditions. Dodine, dichlorprop and dinoseb were found to elute with little retention and therefore a different HPLC system would be required for these pesticides. By employing gradient elution, although losing the separation of certain pesticides, the majority of the compounds can be separated on a single system (Fig. 1) enabling the possibility of cleaned-up extracts to be screened for the possible presence of the listed pesticides. Fig. 2 shows the pesticides (other than organochlorine and organophosphorus compounds) with sufficient absorbance to be determined at the EEC limit.

TABLE III
RELATIVE ELUTION ORDER ON OCTADECYLTRICHLOROSILANE-BONDED SILICA

Dodine	
Dichlorprop	
Dinoseb	
Dimethoate	Compounds not eluted with 75% acetonitrile in water.
Phosphamidon	
Atrazine	Amitrole
Demeton-S-methyl	Omethoate
Propoxur	
Carbaryl	
Thiram	
Paraoxon	Pesticides with low UV absorbance characteristics not included
Formothion	
Captan	
Azinphos-methyl	γ -HCH
Chloroxuron	Demeton-S-methyl sulphone
Parathion-methyl	Oxydemeton-methyl
Endosulfan α	TEPP
Folpet	Trichlorphon
Barban	Camphechlor
Fenitrothion	
Malathion	
Azinphos-ethyl	
Parathion	
Chlorfenson	
Chlorobenzilate	
Methoxychlor	
Fenchlorphos	
Binapacryl	
Diallate Z	
Diallate E	
Endosulfan β	
Endrin	
Chlorbenside	

It has been shown that HPLC with UV detection is not suitable alone for multi-residue analysis but when combined with established GLC procedures for organochlorine and organophosphorus pesticides it is a useful adjunct for multi-

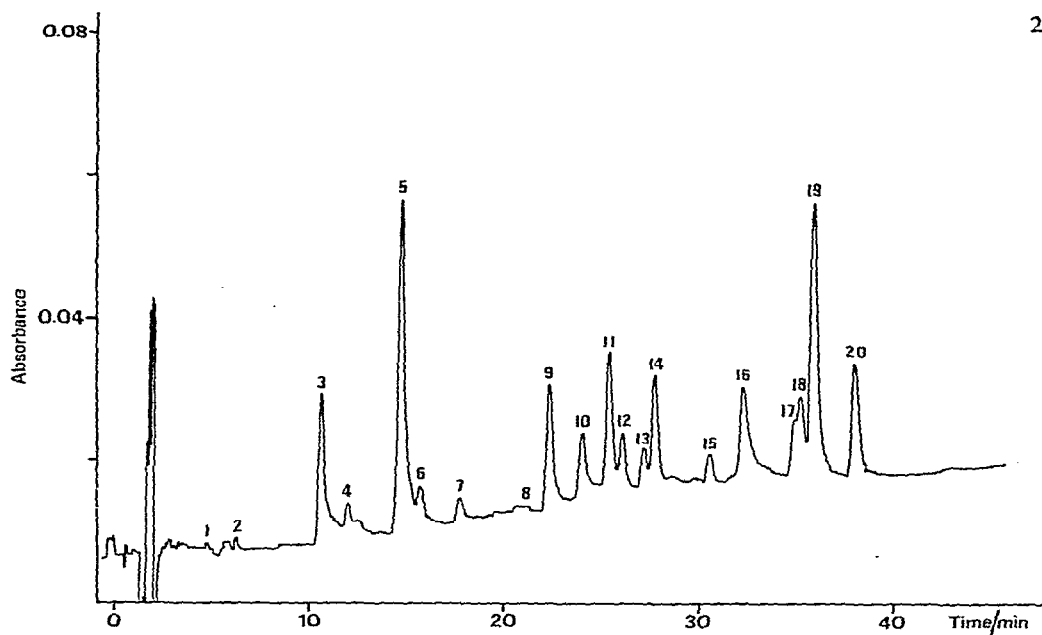


Fig. 1. Separation of pesticides in the EEC Directive. Linear gradient elution from 25% to 75% acetonitrile in water. Flow-rate, 1 ml/min. Detector set to 215 nm. Approximately 100 ng of each compound injected. Peaks: 1 = phthalimide (impurity from 11); 2 = dimethoate; 3 = atrazine; 4 = propoxur; 5 = carbaryl; 6 = 1-naphthol (impurity from 5); 7 = paraoxon + thiram; 8 = captan; 9 = azinphos-methyl; 10 = chloroxuron; 11 = folpet; 12 = barban + malathion; 13 = fenitrothion; 14 = azinphos-ethyl; 15 = parathion; 16 = chlorobenzilate + chlorfenson; 17 = methoxychlor; 18 = fenchlorphos; 19 = binapacryl; 20 = chlorbenside.

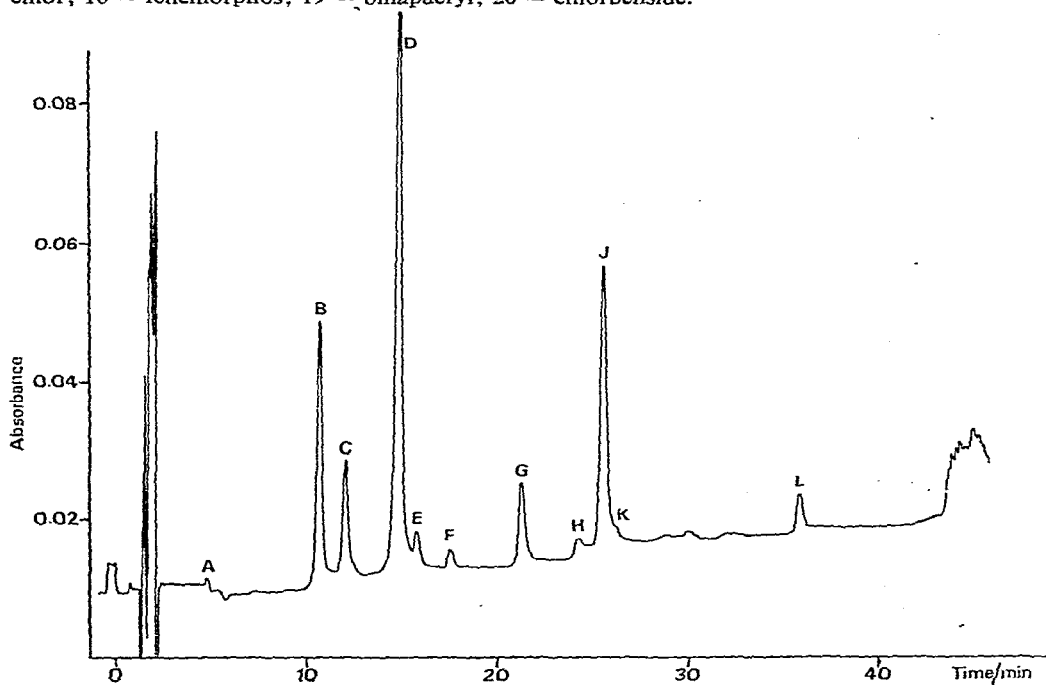


Fig. 2. Range of pesticides at the EEC limit. HPLC conditions as for Fig. 1. Simulation of extract from a 20-g sample concentrated to 2 ml and 10 μ l injected. Peaks (approx. ng injected in parentheses) A = phthalimide (impurity from J) (-); B = atrazine (100); C = propoxur (300); D = carbaryl (120); E = 1-naphthol (impurity from D) (-); F = thiram (300); G = captan (1500); H = chloroxuron (20); J = folpet (1/10 EEC limit) (150); K = barban (10); L = binapacryl (30).

residue screening procedures. The next stage will be to examine the degree of sample clean-up required so that fruit and vegetables can be screened for the pesticides in the Directive by a combined GLC-HPLC procedure.

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