Journal of Chromatography, 234 (1982) 249–254
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,277

Note

Reversed-phase high-performance liquid chromatography of pesticides

VI*. Separation and quantitative determination of some rice-field herbicides

PAOLO CABRAS
Centro Regional Agrario Sperimentale, via Alberti 9, 09100 Cagliari (Italy)
and
PAOLO DIANA, MARCO MELONI and FILIPPO M. PIRISI*
Istituto di Chimica Farmaceutica e Tossicologica, via Ospedale 72, 09100 Cagliari (Italy)
(First received February 26th, 1981; revised manuscript received August 3rd, 1981)

Herbicides of different chemical natures can be exploited to control several rice infesting weeds, *e.g.*, Bentazon (I), 2,4-D (II), MCPA (III), Propanil (IV), Molinate (V) and Drepamon (VI) attack weeds such as *Scirpus mucronatus*, different *Alisma (plantago* and *lanceolata)*, *Echinochloa (crusgalli* and *colonum)* and *Cyperus flavescens*[6]. Although other herbicides can be used, the above mentioned are the most effective under our environmental conditions[7].

These herbicides have been determined by several analytical procedures[8]; recently some high-performance liquid chromatographic (HPLC) analyses have been reported[9]. However, almost all of these methods have been developed for standards and not for environmental samples or for mixtures involving more than two herbicides. Furthermore, only normal phase liquid chromatography has been used.

Since the compounds I–VI are water pollutants, we thought that reversed-phase liquid chromatographic (RPLC) columns should provide the best tool for their analysis. Therefore we have developed a procedure which allows the direct simultaneous separation and determination on a RP-8 column of these herbicides together with widely used insecticides such as Phentoate (VII) in rice-field waters.

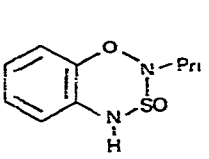
EXPERIMENTAL

Chemicals

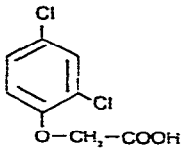
Propanil (3′,4′-dichloropropianilide), MCPA [(4-chloro-*o*-tolyloxy)ethanoic acid] and 2,4-D [(2,4-dichlorophenoxy)ethanoic acid] were AnalGrade standards (Pestanol®, ⩾99.0%; Hoechst, Milan, Italy). Bentazon [3-isopropyl-(1H)-benzo-2,1,3-thiadiazin-4-one-2,2-dioxide], ⩾99.0%, Molinate [S-ethyl N,N-hexamethylenethiocarbamate], ⩾97.0%, Drepamon [S-benzyl di-*sec.*-butylthiocarbamate],

* For parts I–V; see refs. 1–5.

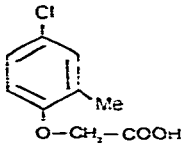
0021-9673/82/0000–0000/$02.75 © 1982 Elsevier Scientific Publishing Company



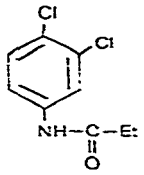
I



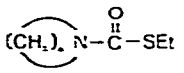
II



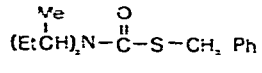
III



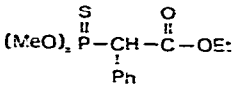
IV



V



VI



VII

Et = Ethyl
Me = Methyl
Ph = Phenyl
Pri = Isopropyl

≥98.5%, and Phentoate [S-α-ethoxycarbonylbenzyl O,O'-dimethyl phosphorodithioate], ≥99.0%, were Analytical Standards kindly donated by BASF Agritalia, SIPCAM and Montedison respectively. Acetonitrile and methanol were HPLC grade solvents purchased from E. Merck (Darmstadt, G.F.R.) and Carlo Erba (Milan, Italy). Water was twice distilled and filtered through a Millipore apparatus before use. Ethyl acetate employed for extractions from water was Anal-Grade (E. Merck).

TABLE I

UV SPECTRA OF THE HERBICIDES IN ACETONITRILE

Herbicide	λ_1 *(nm)*	$\varepsilon \cdot 10^{-3}$	λ_2 *(nm)*	$\varepsilon_2 \cdot 10^{-3}$	ε_{220}
1 Bentazon	221.5	30.7	230.0	11.0	15.4
2 2,4-D	200.0	46.8	227.0	11.2	10.4
3 MCPA	198.0	42.4	227.0	11.2	9.0
4 Propanil	210.0	30.8	250.0	20.2	10.2
5 Molinate	198.0	12.7	—	—	7.6
6 Phentoate	197.0	38.5	220.0	13.8	13.8
7 Drepamon	195.0	30.7	—	—	11.7

TABLE II

RETENTION TIMES OF PESTICIDES UNDER DIFFERENT CONDITIONS

Herbicide	*Water–methanol*						*Water–acetonitrile*					
	RP-2		*RP-18*		*RP-8*		*RP-2*		*RP-18*		*RP-8*	
	0:100	*15:85*	*60:40*	*30:70*	*90:10*	*30:70*	*10:90*	*30:70*	*40:60*	*30:70*	*40:60*	*30:70*
1 Bentazon	1.74	2.07	1.45	1.44	2.85	1.54	1.45	1.70	1.35	1.33	1.45	1.44
2 2,4-D	2.67	2.07		1.44	3.96	1.54	2.26	1.70	1.35	1.33	1.68	1.78
3 MCPA	2.67	2.07	>40.00	6.90	5.64	1.54	2.48	1.70	1.35	3.81	1.75	1.86
4 Propanil	2.67	3.64	>40.00	6.90	>40.00	5.97	2.68	3.65	5.33	3.81	4.88	3.75
5 Molinate	2.80	4.00	>40.00	8.68	>40.00	7.73	2.95	5.52	7.77	5.31	8.67	5.22
6 Drepamon	3.03	5.07	>40.00	18.41	>40.00	20.17	3.52	8.41	>40.00	15.25	17.47	8.44
7 Phentoate	2.80	3.84	>40.00	10.40	>40.00	6.46	2.95	4.29	11.60	5.99	5.88	4.42

Apparatus

A Varian 5020 liquid chromatograph equipped with a Valco AH 20 automatic injector (loop 50 μl), UV/visible Varichrom detector, CDS 111 L data system and Varian 9176 recorder (1 mV/full scale) was employed. From the UV spectra of the pesticides (Table I), the best wavelength for their simultaneous determination was found to be 220 nm.

Chromatography

Merck columns Hibar® RP-2, RP-8 and RP-18 (250 × 4.2 mm I.D., 10 μm) were employed. The mobile phases were water–methanol, water–acetonitrile and mixtures of 0.2 *M* acetic acid–sodium acetate, pH 3.0, 3.5 or 4.0, and 0.067 *M* phosphate pH 5.0, 6.0 or 7.0, buffers in acetonitrile in different ratios.

RESULTS AND DISCUSSION

A preliminary investigation carried out on RP-18, RP-2 and RP-8 columns and under isocratic elution conditions showed that the herbicides could be divided into two main groups, depending on their retention times. While Bentazon, 2,4-D and MCPA (group A) were quickly eluted, Propanil, Molinate and Drepamon (group B) had longer retention times. This behaviour was observed reproducible and was found to be independent of the eluting mixtures and columns (see Table II).

A separation of the pesticides was obtained with water–acetonitrile (40:60 to 30:70) on a RP-8 column, and an improved selectivity was achieved using buffers instead of water in the eluting mixtures. With buffers of pH 7.0, 6.0 or 5.0 no significant changes in the separation of the group A peaks were observed; however, with buffers of pH 4.0 a good separation was obtained and carboxylic acids showed sharper peaks (according to their pK_a values[8]).

A further 5% increase in buffer content of the eluting mixture resulted in optimum separations. Significant changes were observed on decreasing the buffer pH values (see Table III) from pH 4.00 to pH 3.50; below the latter value the chromatogram remained virtually unchanged (see Fig. 1).

The detection limits for injection of a 50-μl non-concentrated water sample (0.004 AU) ranged between 0.01 and 0.03 ppm (see Table IV).

TABLE III

INFLUENCE OF pH ON RETENTION TIMES ON A RP-8 COLUMN

Herbicide	*Retention time (min)*		
	Buffer, pH 4 (35:65)	*Buffer, pH 3.5 (35:65)*	*Buffer, pH 3 (35:65)*
Bentazon	1.75	1.77	2.19
2,4-D	1.92	2.07	2.66
MCPA	2.01	2.26	2.87
Propanil	4.14	4.12	4.16
Molinate	4.86	4.82	4.89
Phentoate	6.26	6.22	6.31
Drepamon	10.99	10.97	11.17

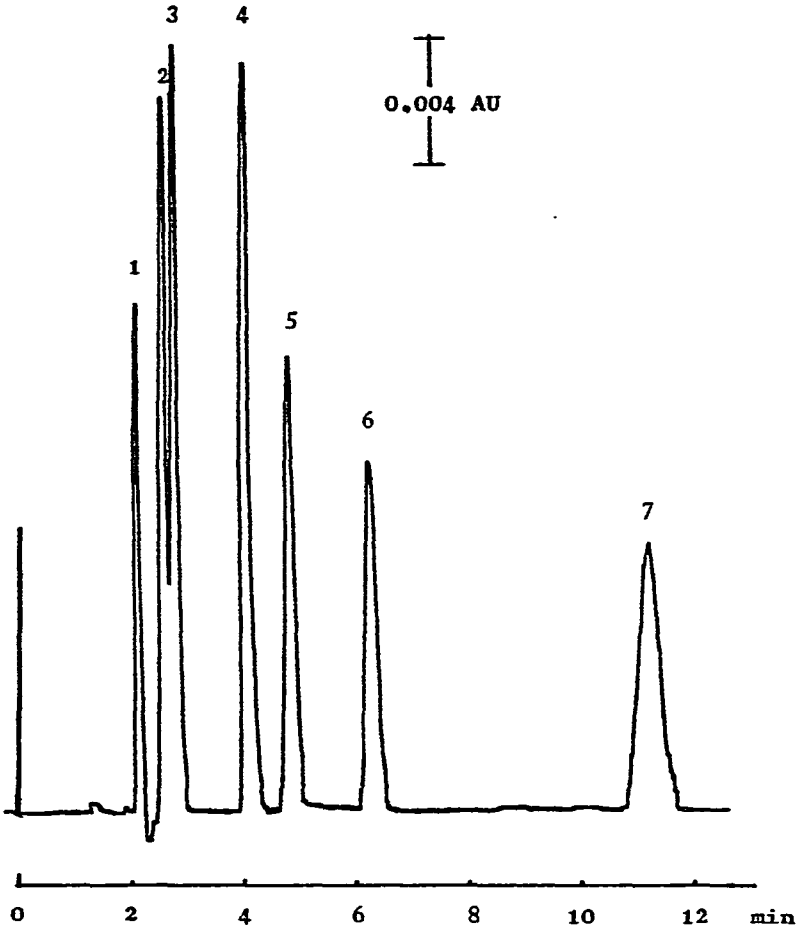


Fig. 1. Chromatogram of heribicides (see Table I) on a RP-8 column. Eluent: buffer, pH 3-acetonitrile (35:65); flow-rate 1 ml/min. Detector: UV at 220 nm. Peaks: 1 = Bentazon; 2 = 2,4-D; 3 = MCPA; 4 = Propanil; 5 = Molinate; 6 = Phentoate; 7 = Drepamon.

TABLE IV

DETECTION LIMITS AT 220 nm

Pesticide	*Detection limit (ppm)*
Bentazon	0.01
2,4-D	0.03
MCPA	0.03
Propanil	0.03
Molinate	0.03
Phentoate	0.03
Drepamon	0.03

ACKNOWLEDGEMENTS

We are indebited to BASF Agritalia (Milan, Italy), SIPCAM (Milan, Italy) and Montedison (Milan, Italy) for gifts of herbicides, and to Dr. Lai and the Consorzio di Bonifica del Campidano di Oristano for their cooperation. We also thank Dr. Franco Cozzi for helpful discussion. This work was made possible by the financial support of Regione Autonoma della Sardegna and has been carried out within the Oriented Project No. 8 of CNR (Italian Research Council).

REFERENCES

1 M. Meloni, F. M. Pirisi and P. Cabras, *Rend. Seminario Fac. Sci. Univ. Cagliari*, 48 (1978) 293.
2 P. Cabras, M. Meloni and F. M. Pirisi, *J. Chromatogr.*, 176 (1979) 473.
3 P. Cabras, M. Meloni, M. Perra and F. M. Pirisi, *J. Chromatogr.*, 180 (1979) 184.
4 P. Cabras, P. Diana, M. Meloni and F. M. Pirisi, *Riv. Viticol. Enol.*, 32 (1979) 3.
5 P. Cabras, P. Diana, M. Meloni and F. M. Pirisi, *J. Agr. Food Chem.*, in press.
6 N. Rizzotto and D. Rui, in *Diserbo*, Edit. Agricole, Bologna, 1976, pp. 244–247.
7 G. Lai, Consorzio di Bonifica Campidano di Oristano, personal communication, 1980.
8 C. R. Worthing (Editor), *The Pesticide Manual*, The British Crop Protection Council, Croydon, 6th ed., 1979.
9 J. F. Lawrence and D. Turton, *J. Chromatogr.*, 159 (1978) 207; and references cited therein.
10 Italian Health Department Act January 6, 1979, *Gazzetta Ufficiale Italian Republic*, February 8, 1979, p. 1282.