[17] CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 66th ed., 1985.
[18] E. Keshavarz and S. Nakai, Biochem. Biophys. Acta, 576 (1979) 269–279.
[19] V.P. Shanbhag and C.G. Axelsson, Eur. J. Biochem., 60 (1975) 17–22.
[20] Min Lu, P.-Å. Albertsson, G. Johansson and F. Tjerneld, J. Chromatogr. A, 668 (1994) 215–228.
[21] J.A. Asenjo, A.S. Schmidt, F. Hachem and B.A. Andrews, J. Chromatogr. A, 668 (1994) 47–54.
[22] C. Hassinen, K. Köhler and A. Veide, J. Chromatogr. A, 668 (1994) 121–128.
[23] H.-O. Johansson, G. Karlström, B. Mattiasson and F. Tjerneld, Bioseparation, (1995) in press.
[24] H.A. McKenzie, Milk Proteins: Chemistry and Molecular Biology, Academic Press, New York, 1971.
[25] T. Peters, Adv. Protein Chem., 37 (1985) 161–245.
[26] L. Piculell, S. Nilsson, L. Falck and F. Tjerneld, Polym. Comm., 32 (1991) 158–160.
[27] G. Zubay, Biochemistry, Macmillan Publishing Company, New York, 2nd ed., 1988, p. 88.
[28] P.G. Righetti and T. Caravaggio, J. Chromatogr., 127 (1976) 1–28.
[29] M. Wahlgren, Ph.D. Thesis, University of Lund, Lund, 1992.
[30] C. Tanford and M.L. Wagner, J. Am. Chem. Soc., 76 (1954) 3331–3336.
[31] H. Theorell and Å. Åkesson, J. Am. Chem. Soc., 63 (1941) 1818–1820.
[32] T. Imoto, L.N. Johnson, A.C. North, D.C. Phillips and J.A. Rupley, in P.D. Boyer (Editor), The Enzymes, Vol. 7, Academic Press, New York, 1972, pp. 665–868.
[33] M. Dautrevaux, Y. Bolonger, K. Han and G. Biserte, Eur. J. Biochem., 11 (1969) 267–277.
[34] R.E. Dickerson, T. Takano, D. Eisenberg, O.B. Kallai, L. Samson, A. Cooper and E. Margoliash, J. Biol. Chem., 246 (1971) 1511–1535.

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JOURNAL OF CHROMATOGRAPHY A

Separation of phenylurea pesticides by ion-interaction reversed-phase high-performance liquid chromatography Diuron determination in lagoon water

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Abstract

An ion-interaction HPLC method is developed for the separation of the phenylurea herbicides asulam, diuron, isoproturon, linuron and monuron. C_{18} was used as stationary phase and octylammonium phosphate as ion-interaction reagent, in the presence of methanol or acetonitrile as organic modifier.

Detection limits lower than 9 μg/l can be obtained without preconcentration steps. The method was applied to the analysis of diuron in a sample of lagoon water. Using liquid–liquid extraction, a diuron concentration of 42 μg/l was found.

1. Introduction

A reversed-phase ion-interaction chromatographic method is presented for the separation of the phenylurea herbicides asulam, diuron, fenuron, isoproturon, linuron and monuron.

Phenylurea derivatives, used as soil sterilants, find their main utilization in weed control. Linuron is preferentially used as a pre-emergence selective herbicide for cereals, vegetables and small fruit crops; fenuron and monuron are mainly used for general weed control in noncrop land, while diuron is principally recommended for the control of aquatic weeds and algae in farm ponds, dugouts, irrigation banks, ditches and canals [1,2].

Phenylurea herbicides are photochemically unstable [3,4] and little information is available about their long-term toxic effects and mutagenicity [5]. Only monuron has been implicated for possible carcinogenity.

These herbicides are water soluble and from the soil they can easily migrate to crops and enter the food chain. Depending on the particular rainfall conditions and soil properties, the herbicides can also reach ground waters where, due to the absence of microbial activity, degradation processes are very slow and accumulation phenomena can easily lead to toxic levels [6].

The Commission of the European Community, Drinking Water Directive 80/778 (CEC-DWD) indicates a maximum amount of 0.5 μg/l

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of total herbicide and 0.1 μg/l for each constituent. No concentration is given for other surface waters.

In the literature HPLC and GC methods have been described for the determination of phenylurea herbicides in surface waters as well as in crops and vegetables [7–11].

The sensitivity required for drinking water analysis is generally reached through preconcentration steps. A variety of adsorbents–such as C_8, C_{18}, cyclohexyl, bonded-silica or styrene–divinyl–benzene based size-exclusion phases [11–20]–are used for solid-phase extraction (SPE) often in combination with on-line enrichment or column switching [11,21–29]. Graphitized carbon-black Carbopack cartridges are also employed [30]. Liquid–liquid extraction [31] and post-column derivatization with *o*-phthalaldehyde–2-mercaptoethanol [32] have been used and examples can be found of HPLC with particle-beam [14,16,33] and thermospray mass spectrometric detection [7,27].

An ion-interaction method described for the determination of asulam in apples makes use of sodium cholate as the ion-interaction reagent in the presence of tetramethylammonium hydrogensulfate, triethylamine, acetic acid and methanol [34].

This paper presents the development and the optimization of a new and sensitive ion-interaction HPLC chromatographic method for the separation and determination of asulam, monuron, diuron, fenuron, linuron and isoproturon. The separation of thiourea, phenylurea and ethylenethiourea, which can be regarded as their base-structures, is also studied.

2. Experimental

2.1. Apparatus

The chromatographic analyses were carried out with a Merck-Hitachi Lichrograph chromatograph Model L-6200 (Tokyo, Japan), equipped with a two-channel D-2500 Chromato-integrator, interfaced with a UV–Vis detector L-4200 and a L-3720 conductivity detector with a temperature control unit from the same firm.

Spectrophotometric determinations were performed with a UV–Vis Hitachi (Tokyo, Japan) Model 150-20 spectrophotometer.

pH measurements were performed with a Metrohm (Herisau, Switzerland) 654 pH meter equipped with a combined glass-calomel electrode.

2.2. Chemicals and reagents

Ultrapure water from Millipore (Milford, MA, USA) Milli-Q was used for the preparation of solutions. Methanol and acetonitrile LiChrosolv gradient grade solvents and thiourea were Merck (Darmstadt, Germany) reagents. Octylamine, ethylenethiourea, phenylurea and orthophosphoric acid were Fluka (Buchs, Switzerland) analytical grade chemicals. Asulam, diuron, fenuron, isoproturon, linuron, monuron were analytical grade LabService Analytica (Anzola dell Emilia, Bologna, Italy) chemicals. All other chemicals were Carlo Erba (Milano, Italy) analytical reagents.

2.3. Chromatographic analysis

Reversed-phase ion-interaction HPLC was employed according to methods already used in this laboratory for separations of anions and amines [35,36].

A 5-μm ODS-2 Spherisorb (Phase Separation, Deeside, UK) column was used, equipped with an RP-18 (5 μm) guard column (Lichrospher, Merck, Darmstadt, Germany). The mobile phase was an aqueous or an aqueous-organic solution of *n*-octylammonium–*o*-phosphate. It was prepared by adding the organic solvent to the amount of octylamine weighted to prepare a 5.0 m*M* solution. Orthophosphoric acid was added to obtain a pH of 6.4 ± 0.2. The pH thus obtained for the aqueous-organic solution was also reported as an "operational" pH value [37].

The chromatographic system was conditioned by passing the eluent through the column until a stable baseline signal was obtained; a minimum

of 1 h was necessary at flow-rate of 1.0 ml/min. After use, the column was washed and regenerated with flowing water (0.50 ml/min for 15 min), water–methanol (50:50, v/v) or water–acetonitrile (50:50, v/v) (0.50 ml/min for 1 h), and then with 100% methanol or acetonitrile (0.50 ml/min for 5 min).

Zero retention time (t_0) was evaluated through injection of sodium nitrate solutions (15.0 mg/l) and the conductometric detection of the unretained sodium ion.

Spectrophotometric detection at 240 nm was employed for herbicide analysis.

Our results fit the model according to which the ion-interaction reagent contained in the mobile phase is bound onto the surface of the stationary phase through adsorption and electrostatic forces, giving rise to an electrical double layer. The interaction properties of the original reversed-phase packing material are therefore modified. The modified surface is able to simultaneously retain anions and cations [35,38].

2.4. Preparation of the real sample

The lagoon sample was collected in 2.0-l pyrex glass bottles previously washed with 0.2 *M* hydrochloric acid and repeatedly rinsed with ultrapure water. During sampling bottles were rinsed twice with the lagoon water, then filled and tightly capped. The entire sample was filtered through Millipore 0.45-μm filters and stored at 4°C. Analysis was performed within three days after sampling.

A 1500-ml volume of sample was brought to pH 2.50 with hydrochloric acid and filtered through a 0.22-μm nylon 66 membrane filter. A 100-g amount of $NaClO_4$ was added, and then the sample was extracted three times with 40.0-ml aliquots of dichloromethane. The combined extracts were dehydrated with sodium sulfate, concentrated on a Rotovapor at 25°C under vacuum, evaporated to dryness under a stream of nitrogen, and subsequently diluted to a final volume of 1.5 ml with the mobile phase.

3. Results and discussion

Separation of thiourea, phenylurea and ethylenethiourea was achieved (see Table 1) using a 5.0 mmol/l aqueous solution of octylammonium phosphate as the mobile phase. The separation of the phenylurea pesticides required the addition of organic modifier to perform the elution within reasonable analysis times (without modifier elution times longer than 100 min were obtained). Two series of experiments were carried out with different amounts of methanol or acetonitrile, respectively. The capacity factors obtained are reported in Table 1 and Fig. 1 shows the ln k' values [$k' = (t_R - t_0)/t_0$, where k' is the capacity factor, t_R and t_0 the retention time and zero retention time, respectively] as a function of methanol concentration. The plots can be fitted by straight lines, the slopes of which are very close for all analytes considered. This result suggests that the dependence of retention time on methanol concentration does not seem to be predominantly correlated to the chemical structures of the analytes. This is in agreement with results previously found [39] and will be later discussed.

The results collected in Table 1 show that the optimal concentration of organic modifier which assures a good resolution together with short analysis time is 55% of methanol (at flow-rate of

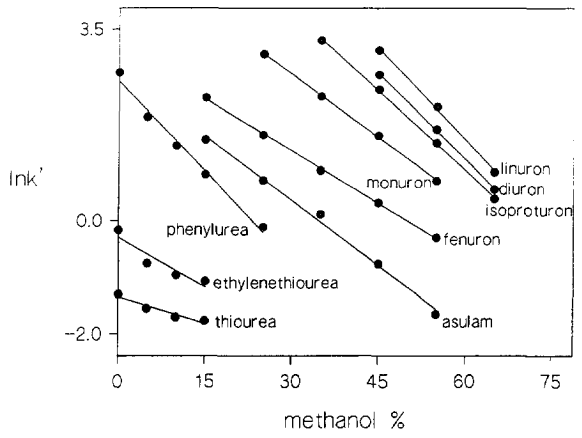

Fig. 1. Plots of ln k' vs. methanol percentages in the mobile phase.

Table 1
Capacity factors of the analytes as function of the organic modifier

Analyte	Mobile phase															
	Water–acetonitrile (65:35) (flow-rate 0.8 ml/min)	Water–methanol (45:55) (flow-rate 1.5 ml/min)	Aqueous 5.0 m*M* *n*-octylammonium–*o*-phosphate (flow-rate 0.7 ml/min)	5.0 m*M* *n*-Octylammonium–*o*-phosphate and acetonitrile at different percentages (flow-rate 0.8 ml/min)					5.0 m*M* *n*-Octylammonium–*o*-phosphate and methanol at different percentages (flow-rate 1.5 ml/min)							
				5	10	15	25	35	5	10	15	25	35	45	55	65
Diuron	11.34	7.70	–[b]	–[b]	–[b]	–[b]	–[b]	5.82	–[b]	–[b]	–[b]	–[b]	–[b]	14.30	5.20	1.76
Monuron	5.44	3.34	–[b]	–[b]	–[b]	–[b]	5.72	2.42	–[b]	–[b]	–[b]	20.84	9.62	4.66	2.03	*[a]
Linuron	21.15	11.16	–[b]	–[b]	–[b]	–[b]	–[b]	11.34	–[b]	–[b]	–[b]	–[b]	–[b]	22.49	7.97	2.38
Isoproturon	10.79	6.48	–[b]	–[b]	–[b]	–[b]	–[b]	5.26	–[b]	–[b]	–[b]	–[b]	26.83	10.87	4.04	1.48
Fenuron	1.19	1.52	–[b]	–[b]	–[b]	4.29	1.74	0.94	–[b]	–[b]	9.43	4.71	2.47	1.38	0.73	*[a]
Asulam	*[a]	*[a]	–[b]	–[b]	–[b]	1.83	0.52	0.23	–[b]	–[b]	4.31	2.06	1.12	0.46	0.19	*[a]
Thiourea	*[a]	*[a]	0.27	0.21	0.18	0.17	*[a]	*[a]	0.25	0.22	0.19	0.16	0.14	*[a]	*[a]	*[a]
Ethylenethiourea	*[a]	*[a]	0.84	0.47	0.38	0.34	*[a]	*[a]	0.58	0.43	0.38	0.25	0.21	*[a]	*[a]	*[a]
Phenylurea	*[a]	*[a]	14.88	6.58	3.85	2.30	0.89	*[a]	10.02	6.24	4.27	2.40	1.38	*[a]	*[a]	*[a]

[a] $k' = 0$.
[b] $k' > 30$.

1.5 ml/min) or 35% of acetonitrile (at flow-rate of 0.8 ml/min). Based on the absorbance/wavelength spectra recorded for the investigated analytes a detection wavelength of 240 nm was chosen.

Fig. 2 shows typical separations obtained for a mixture of the analytes (0.10 mg/l each) with *n*-octylammonium–*o*-phosphate as the interaction reagent in the presence of methanol (Fig. 2A) and acetonitrile (Fig. 2B).

Some suggestions can be made concerning the retention mechanism. As mentioned, ethylenethiourea, thiourea and phenylurea are separated (Table 1) with a mobile phase of an aqueous solution of *n*-octylammonium–*o*-phosphate. In such conditions, retention is likely due to ion-interaction mechanisms, based on the dynamic modification that the octylammonium–*o*-phosphate induces onto the surface of the stationary phase. On the other hand, in the separation of phenylurea pesticides, the addition of organic modifier was necessary in order to obtain analysis times of the order of 30 min. In these conditions, in order to distinguish if the retention process of phenylurea pesticides is really due to ion-interaction mechanisms and not to a conventional reversed-phase partition, chromatographic runs were performed in reversed-phase mode, i.e. by using a mobile phase of the same composition of methanol (55%) or acetoni-

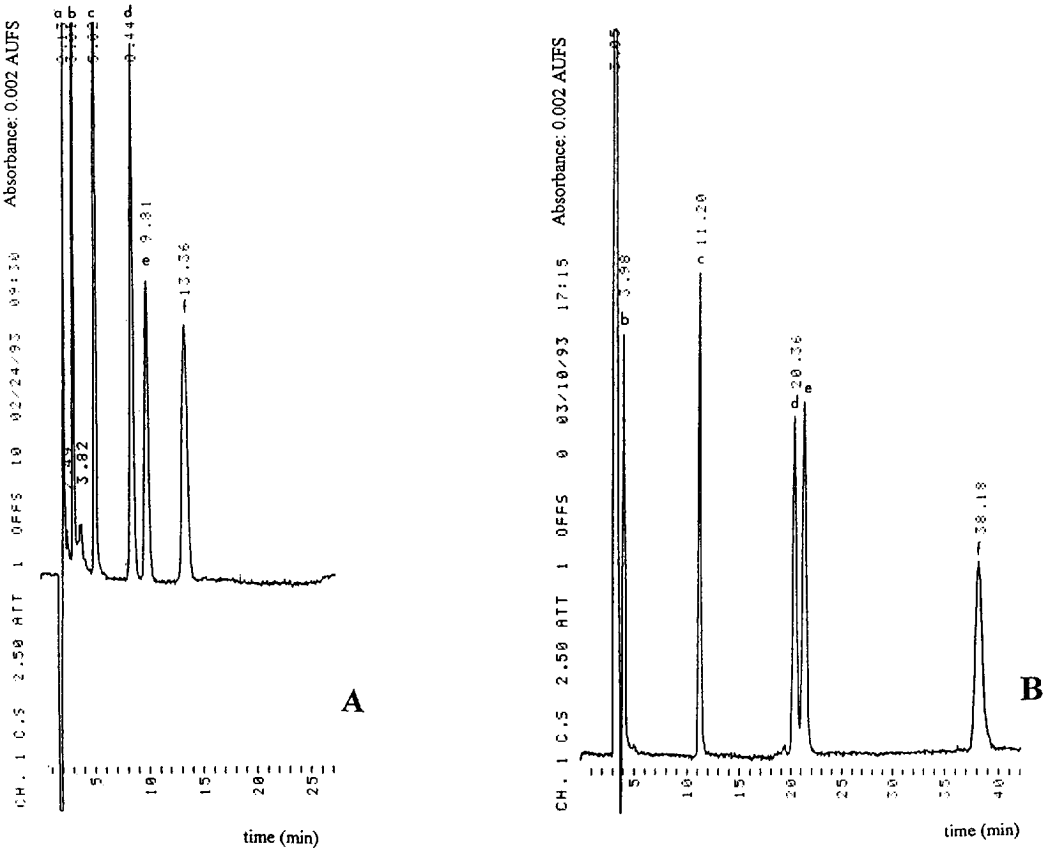

Fig. 2. Elution of the standards under optimized conditions. Stationary phase: Phase Separation ODS-2 Spherisorb, 250 × 4.6 mm I.D., 5 μm, endcapped. Spectrophotometric detection at 240 nm. Mobile phase: (A) 5.0 mmol/l octylammonium phosphate in water–methanol (45:55, v/v), pH 6.4, flow-rate: 1.5 ml/min; (B) 5.0 mmol/l octylammonium phosphate in water–acetonitrile (65:35, v/v), pH 6.4, flow-rate 0.8 ml/min. Peaks: a = asulam, b = fenuron, c = monuron, d = isoproturon, e = diuron, f = linuron.

trile (35%) as in the optimized separations presented in Fig. 2A and 2B, but without the presence of octylammonium–*o*-phosphate. As expected on the basis of their molecular structures, the analytes considered are also separated in reversed-phase mode, but retention times are different (Table 1) and resolution and sensitivity are generally poorer with respect to the elution with the mobile phase containing the ion-interaction reagent.

The result confirms that ion-interaction mechanisms are indeed taking place and the similar slopes of the $\ln k'$ vs. methanol concentration plots (Fig. 1) obtained for the different analytes can be ascribed to the predominant effect that the organic solvent exerts on the moiety adsorbed onto the surface of the stationary phase (and which affects all the analytes in the same way) rather than on the single structure of each analyte.

Calibration plots obtained in the concentration range 1.0–100.0 μg/l indicate a good linearity. From sensitivity data (S, expressed as the peak area given by the integrator for a 1.0 μg/l solution) and evaluation in the chromatogram of a peak area (a) corresponding to an average signal-to-noise ratio of 3, the limits of detection (LOD = a/S μg/l) for each analyte were evaluated. Detection limits were found to be lower than 9.0 μg/l for all analytes investigated. These concentrations are higher than those required for drinking water but are of the same order or lower than those generally reported for surface water, which range between 0.1 and 30 μg/l [16].

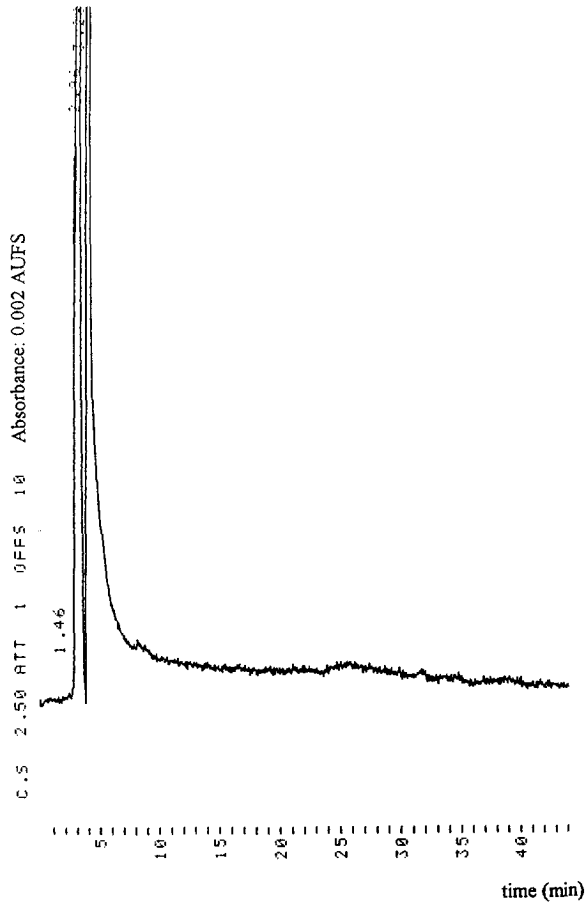

Fig. 3. Chromatographic analysis of a sample of lagoon water. Experimental conditions: Phase Separation ODS-2 Spherisorb column, 250 × 4.6 mm I.D., 5 μm; mobile phase: 5.0 mmol/l octylammonium phosphate in water–acetonitrile (65:35, v/v), pH 6.4, flow-rate 0.8 ml/min; spectrophotometric detection at 240 nm.

3.1. Application to real sample

The method was applied to the analysis of a sample of lagoon water collected in the tidal marsh of Palude di Cona (in the north-east of Venice lagoon) suspected to contain diuron, which is largely employed in river and sea waters for control of algal growth. A preliminary chromatographic run performed on a filtered sample of water under the optimized chromatographic conditions (Fig. 3) permits us to exclude the presence of diuron at a concentration close to or higher than the detection limit of the method (7 μg/l for diuron) and at the same time shows a very low matrix interference in the time window of the herbicide considered. It must in fact be considered that the ion-interaction technique is characterized by selective properties towards non-ionizable or high-molecular-mass species, properties which are particularly advantageous when dealing with complex matrices.

A preconcentration step was then performed for the lagoon water sample. Taking into account