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REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF NITROPHENOLS

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SUMMARY

The reversed-phase ion-pair high-performance liquid chromatography of nitrophenols, using hexadecyltrimethylammonium as the pairing ion, is described. It is shown that with this system the parameters can be easily adjusted to give optimal chromatographic conditions

Another advantage of this technique is that it permits a specific detection of these compounds at longer wavelengths with a good sensitivity. At these wavelengths chromatograms of blank apple extracts are completely free of interferences The efficiency of the extraction of the nitrophenols from water samples is satisfactory and concentrations of 1 μg/l can be easily detected.

INTRODUCTION

Nitrophenol derivatives such as 4,6-dinitro-2-cresol (DNOC), 2-*sec.*-butyl-4,6-dinitrophenol (dinoseb) and 2-*tert.*-butyl-4,6-dinitrophenol (dinoterb) are used as herbicides on a variety of crops and can give rise to residues in various foods. They can also leach from the soil by rain into the ground and surface water. Other nitrophenols, which are not in use as pesticides, can also enter the environment, for instance as degradation products from a variety of chemicals or as discharges from dye manufacturing industries and petrochemical refineries[1,2]. Many of these compounds are highly toxic[3] and some of them are listed as priority pollutants by the U.S. Environmental Protection Agency[4]. Therefore it is necessary to be able to determine their concentration in various matrices, such as plant material, soil and water samples.

Various methods for the determination of nitrophenols by means of gas chromatography have been described. In all these methods the compounds were derivatized in order to increase their volatility, for which purpose various derivatization procedures were used. Conversion into ethyl or methyl ethers with diazoethane[5,6] or diazomethane[7,8] has been described, as well as acetylation with acetic anhydride[2]. Nitrophenols may also be quantitated as heptafluorobutyryl derivatives following reduction of the nitro group[1].

High-performance liquid chromatography (HPLC) has proved to be very

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useful in the analysis of polar compounds such as these nitrophenols. Therefore we studied the behaviour of a number of these compounds in different chromatographic systems. Also their absorption spectra were recorded in order to find an optimal detection wavelength with respect to sensitivity and selectivity. Finally, the efficiency of the extraction of these compounds from water samples was determined.

EXPERIMENTAL

Reagents

The nitrophenols were obtained from various commercial sources or as analytical standards from the manufacturers and were used without further purification.

Methanol (zur Analyse) was obtained from Merck (Darmstadt, G.F.R.), glacial acetic acid (AnalaR) from BDH (Poole, Great Britain). Dichloromethane was obtained from Brocacef (Maarssen, The Netherlands) and distilled prior to use.

Hexadecyltrimethylammonium bromide (cetrimide), obtained from Baker (Deventer, The Netherlands), was dissolved in methanol to a concentration of 10 g/l. K_2HPO_4 and NaH_2PO_4 (Baker, reagent grade) were dissolved in water, both to a concentration of 0.25 *M*. Appropriate volumes of these stock solutions of cetrimide and phosphate were mixed with methanol and water to give eluents with the desired concentrations of cetrimide and phosphate. After mixing, the eluents were passed through a 1-μm filter and deaerated ultrasonically.

High-performance liquid chromatography

Analyses were conducted with a Varian 8500 liquid chromatograph, equipped with a Valco loop injector, a Varian Micropak CH-10 column (25 cm × 2 mm I.D.) and Variscan variable-wavelength detector. Eluents used were methanol–water (65·35, v/v), containing 1 % (v/v) of glacial acetic acid or methanol–water (75:25, v/v), containing various concentrations of phosphate and cetrimide. The flow-rate was 30 ml/h and chromatography was carried out at ambient temperature.

UV spectra

The compounds were dissolved to a concentration of 10 μg/ml in methanol–water (65:35, v/v), containing 1 % (v/v) of glacial acetic acid and in methanol–water (75·25, v/v), containing 0.24 % (w/v) of cetrimide and 0.012 *M* of phosphate. From these solutions UV spectra were recorded in a 1-cm cell on a Perkin-Elmer Model 570 spectrophotometer.

Extraction from water samples

Water samples of 100 ml were spiked with 4-nitrophenol, DNOC and dinoseb to a concentration of 50 μg/l. The samples were acidified to pH 1, 4 or 6 and extracted with 20 ml of dichloromethane and the extraction was repeated twice with 10 ml of dichloromethane. At pH 4 the samples were also extracted with 20, 20 and 10 ml and with 10, 10 and 10 ml of dichloromethane. The combined extracts were evaporated to dryness under reduced pressure at 40°C and the residue was transferred to a centrifuge tube with 10 ml of toluene. To this solution was added 1 ml of 0.05 *M* K_2CO_3 solution and, after shaking, 10 μl of the aqueous phase were injected onto the HPLC column, using an ion-pair system and detection at 365 nm.

RESULTS AND DISCUSSION

Ion-pair techniques have been introduced into HPLC during the past decade for tackling otherwise difficult analyses of easily ionized species. The main advantage of this technique is the fact that there are a number of parameters that can be easily adjusted to produce optimal chromatographic conditions[9]. Both the theoretical and experimental aspects of reversed-phase ion-pair HPLC suggest that this is the preferred method for using ion-pair techniques. This approach gives rise to very stable systems, and when surface-active pairing ions such as hexadecyltrimethylammonium (cetrimide) or alkylbenzyldimethylammonium are used, the chromatographic efficiency is at least as good as with non-ion-pair HPLC techniques[10].

For these reasons we studied the chromatographic behaviour of nitrophenols in a reversed-phase system with various concentrations of cetrimide and phosphate in the mobile phase. Also the capacity factors of these compounds were determined in a reversed-phase system with an acidic eluent, in which system the compounds are chromatographed as non-ionized species. The results of these studies are given in Table I, which shows that an increase in the phosphate concentration causes a decrease in the capacity factor, whereas an increase in the cetrimide concentration causes an increase in the capacity factor. These changes in capacity factor are different for all the compounds, so that the separation between the phenols can be

TABLE I

CAPACITY FACTORS (k') FOR NITROPHENOLS WITH VARIOUS SOLVENTS ON A REVERSED PHASE COLUMN (VARIAN CH10)

Solvent systems: I: methanol–water (75·25, v/v); 0·24% (w/v) cetrimide; 0·0125 *M* PO_4^{3-}; II methanol–water (75·25, v/v), 0·24% (w/v) cetrimide; 0·006 *M* PO_4^{3-}, III methanol–water (75·25, v/v), 0·12% (w/v) cetrimide; 0·0125 *M* PO_4^{3-}; IV: methanol–water (75·25, v/v); 0·12% (w/v) cetrimide; 0·006 *M* PO_4^{3-}; V methanol–water (75·25, v/v); 0.48% (w/v) cetrimide; 0.0125 *M* PO_4^{3-}; VI methanol–water (75·25, v/v), 0·48% (w/v) cetrimide; 0.006 *M* PO_4^{3-}; VII: methanol–water (65·35, v/v), 1% (v/v) glacial acetic acid. The PO_4^{3-} concentration is the sum of equal concentrations of NaH_2PO_4 and K_2HPO_4.

Compound	*k'*						
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*
4-Nitrophenol	1·52	1·61	1·09	1.29	1·81	1·87	0·68
3-Nitrophenol	1·65	1·75	1·40	1·53	1·93	2·06	0·82
3,4-Dinitrophenol	1.77	1·88	1·20	1·49	2.19	2·35	0·80
3-Methyl-4-nitrophenol	1·75	1·92	1.38	1.52	2.11	2·20	1·05
2,4-Dinitrophenol	2·42	2·58	1.66	1.91	3·31	3.42	0·91
1-Chloro-2,4-dinitrophenol	2·52	2·67	1.70	1.94	3·23	3·46	0·91
2-Nitrophenol	2·58	2·71	1·88	2.10	3·27	3·52	1·36
2,6-Dinitrophenol	2·65	2.91	1·76	2·05	3·84	4·16	0·68
2,3-Dinitrophenol	2·83	2·98	1·96	2·13	3·71	3·86	0·57
3-Methyl-6-nitrophenol	3·13	3·32	2·40	2.65	3·88	4·10	2.66
DNOC	3.16	3.33	2.13	2.48	4·50	4·55	2·23
4-Methyl-2-nitrophenol	3·33	3·49	2·61	2.88	4·15	4·30	2.55
2,5-Dinitrophenol	3·36	3·57	2.32	2·58	4.63	4.75	0·98
Dinoseb	6·49	6·73	4·39	5·32	8·88	8·99	9·55
4-Chloro-2-nitrophenol	6.68	6·87	4·96	5·54	8.80	9.09	2·73
Dinoterb	7.54	8·00	5·06	6·02	10·30	10.42	11·27

optimized by adjusting the concentrations of phosphate and cetrimide. This can be illustrated by calculating the number of theoretical plates required for a 98% complete separation of the compounds dinoseb, 4-chloro-2-nitrophenol and dinoterb in the systems III and IV[11]. In system III dinoseb and 4-chloro-2-nitrophenol can be completely separated on a column with less than 2000 theoretical plates, whereas for a complete separation of 4-chloro-2-nitrophenol and dinoterb a column with *ca.* 60,000 theoretical plates is needed. On the other hand, in system IV a column with 3500 plates is sufficient for a complete separation of 4-chloro-2-nitrophenol and dinoterb, but in this system more than 14,000 plates are needed for a complete separation between dinoseb and 4-chloro-2-nitrophenol.

These differences in the influence of the chromatographic conditions on the capacity factor, which sometimes even result in a reversal of the elution order, can be used very well for identification of the compounds.

The elution order with the acidic eluent is completely different from the elution order in the ion-pair system, as can be seen in Table I. With this acidic eluent many of the nitrophenols can be separated, but some of them have capacity factors that are very close to each other. Because this system does not have the flexibility of the ion-pair system for adjustment of the parameters to improve the separation, the latter system is to be preferred.

In order to find an optimal wavelength for detection of the nitrophenols, the absorption spectra of a number of them were recorded in two different solvents, *i.e.* in solvents I and VII (see Table I). A typical example is given in Fig. 1.

In acidic media the nitrophenols have a maximum of absorption at a wavelength of *ca.* 230 nm, with a molar absorptivity between 8000 and 15,000, and a maximum at *ca.* 280 nm with a molar absorptivity in the same range. In neutral media the maximum at the shortest wavelength is the same, but the maximum at *ca.* 280 nm is shifted towards longer wavelengths, while the molar absorptivity does not change substantially. Thus in acidic media a detection wavelength below 300 nm should be chosen, whereas in neutral media a detection wavelength between 360 and 420 nm would also give a good sensitivity.

Detection at longer wavelengths has the advantage of being more specific, as can be seen in Fig. 2, which shows chromatograms of blank apple extracts. Blank apples were extracted with dichloromethane, the dichloromethane was evaporated and the residue was dissolved in methanol–0.02 *M* K_2CO_3 solution (1:1). This solution was filtered through a 0.45 μm filter and an aliquot, equivalent to 1 g of apple, was injected onto the HPLC column. The detection was carried out at 280 nm and at 365 nm. The sensitivity is such that in both chromatograms 100 ng of DNOC would give 50% of full-scale deflection. As can be seen from this figure, the chromatogram is clean at the retention time of the nitrophenols when detection is carried out at 365 nm; when 280 nm is used as the detection wavelength many interfering peaks show up.

Table II lists the recoveries of the extraction of 4-nitrophenol, DNOC and dinoseb from spiked water samples under various conditions. These compounds were chosen because they are likely to be encountered in practice and because they cover the complete polarity range of the nitrophenols investigated; 4-nitrophenol is probably the most polar compound from the nitrophenols, while dinoseb is about the least polar.

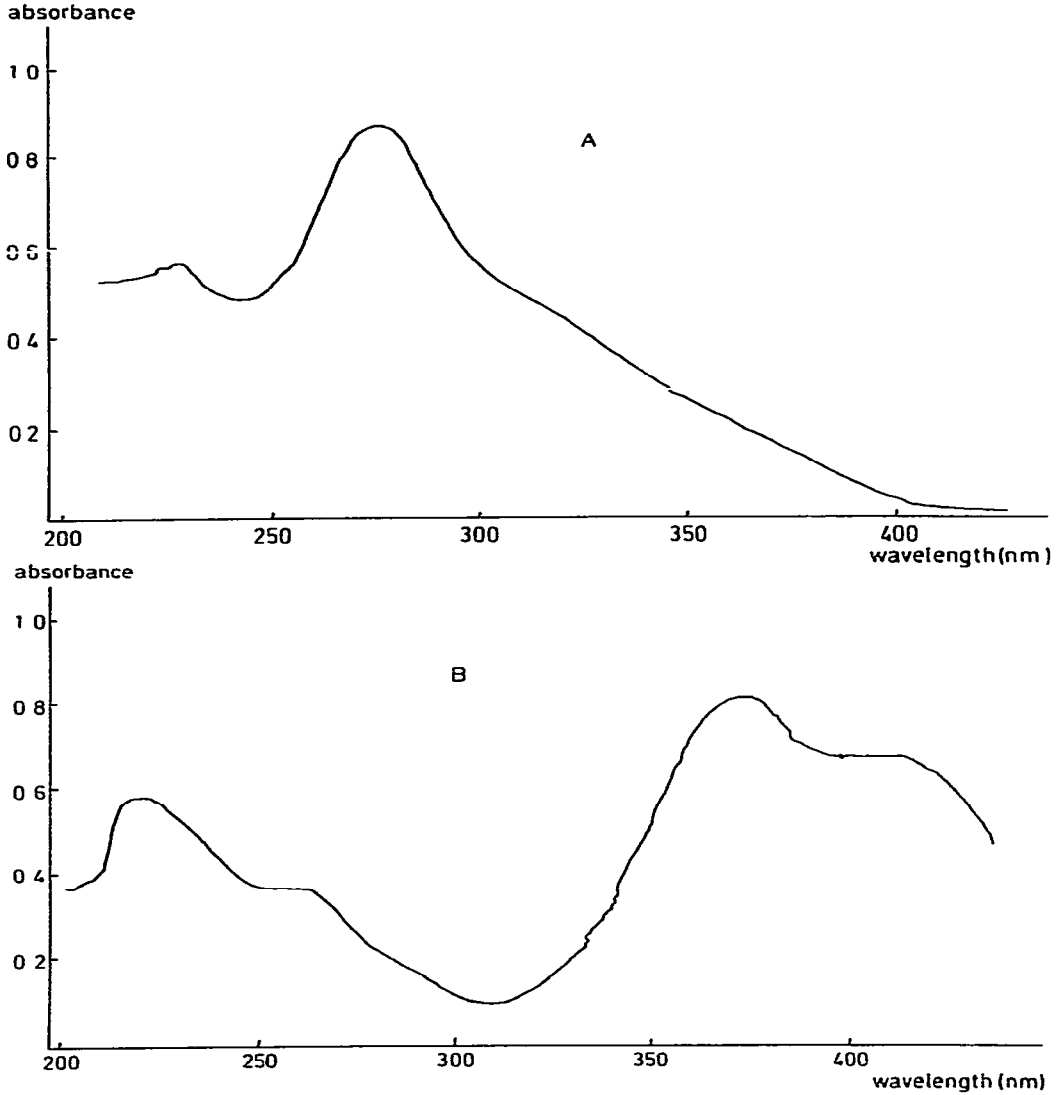

Fig. 1 UV absorption spectra of 10 μg/ml solutions of DNOC in acidic (A) and neutral (B) media

TABLE II
RECOVERY OF 4-NITROPHENOL, DNOC AND DINOSEB FROM WATER SAMPLES UNDER VARIOUS EXTRACTION CONDITIONS

pH	*Dichloromethane used (ml)*	*Percent extracted**		
		4-Nitrophenol	*DNOC*	*Dinoseb*
1	20 + 10 + 10	84	87	96
4	10 + 10 + 10	66	80	106
4	20 + 10 + 10	81	94	95
4	20 + 20 + 10	88	90	103
6	20 + 10 + 10	70	83	104

* Each value is the mean of three determinations

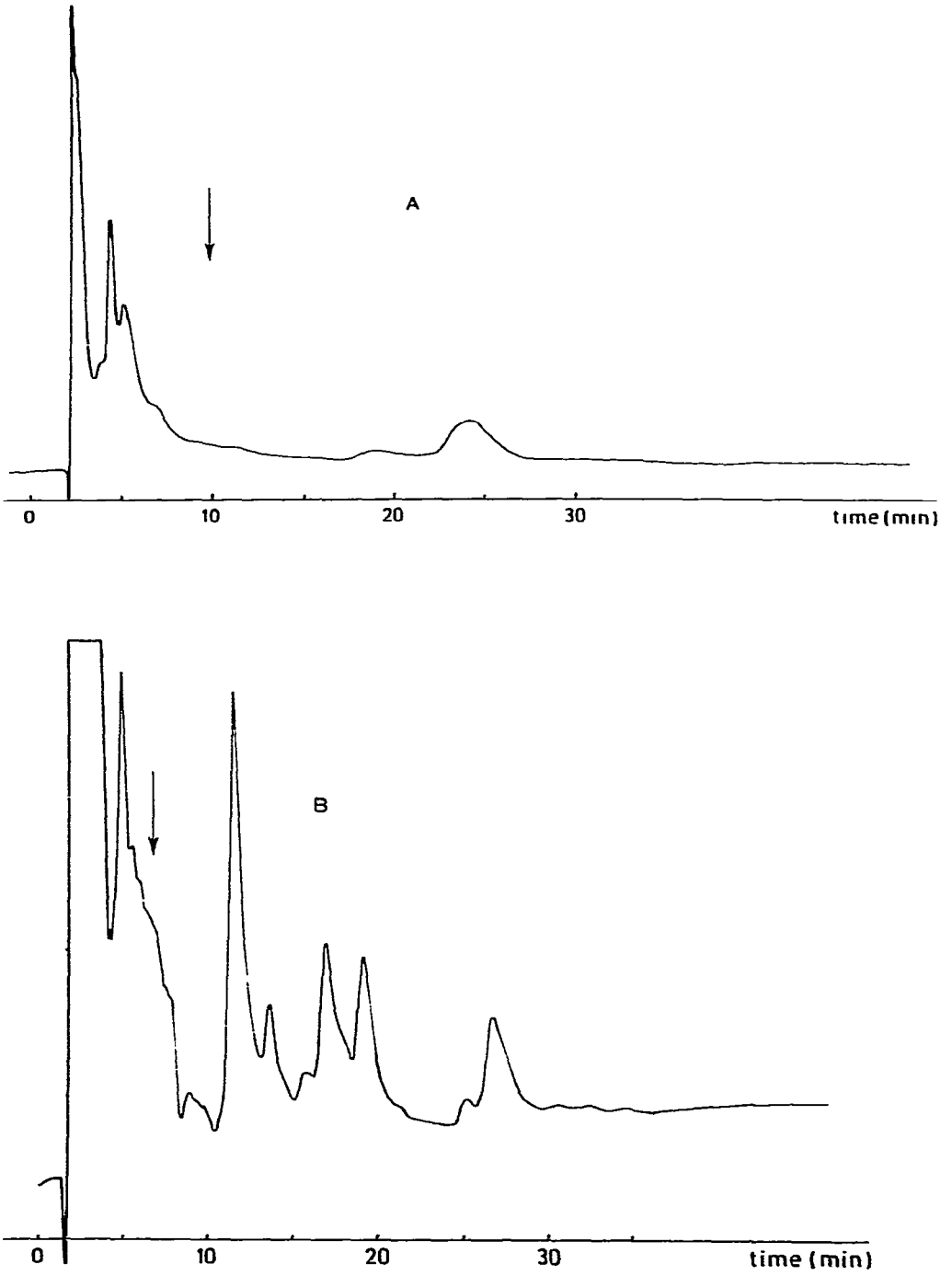

Fig. 2. Chromatograms of blank apple extracts for conditions see text (A) Detection at 365 nm (B) Detection at 280 nm The arrow indicates the retention time of DNOC.

In general, the recoveries of these compounds from water samples are quite satisfactory. Only at pH 6, and when the extraction is carried out with three times 10 ml of dichloromethane, are the recoveries somewhat low for 4-nitrophenol. At pH values below 6 and when a larger volume of dichloromethane is used, the recoveries are good.

A water sample, obtained from the river Rhine, was extracted with dichloromethane and analyzed on HPLC as described. No peak was detectable that could be attributed to one of the nitrophenols. The limit of detection in this experiment was 1 μg/l. By using a water sample of 1 l and concentrating this to a final volume of 1 ml, of which 50 μl are injected, a limit of detection of 0.02 μg/l can probably be reached. However, the U.S. Environmental Protection Agency has set concentration limits of 250 and 100 μg/l for 2- and 4-nitrophenol, respectively, in drinking water[12], so a detection limit of 1 μg/l seems quite adequate.

CONCLUSIONS

Reversed-phase ion-pair HPLC with cetrimide as the pairing ion can be used very well for the separation of nitrophenols. The ion-pair system can be easily adjusted to optimize the separation or to identify the various compounds. Another advantage of this system is the fact that it permits a specific detection at longer wavelengths with a good sensitivity.

The technique seems to be very useful for the determination of nitrophenols in plant materials and in environmental samples.

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