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Note

High-performance liquid chromatography of some hydroxy-substituted aromatic compounds

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To identify and quantitate total organic compounds at trace levels in water generally involves preconcentrations by solvent extraction, continuous liquid–liquid extraction or on-column absorption[1–3]. The disadvantages of such methods are the long sample preparation time, the possibility of incomplete recoveries of the compounds, the necessity of derivatization to make the molecules more volatile and stable for gas chromatographic analysis.

Our study concerns a series of phenolic substances having industrial interest: We have investigated the high-performance liquid chromatographic (HPLC) separation on an RP-18 reversed-phase column of alkyl- and benzophenones and monohydroxy- and dihydroxynaphthalenes. With reversed-phase chromatography it is possible to inject directly aqueous samples, and with sufficiently sensitive detectors any preconcentration process becomes unnecessary.

The electrochemical detector seemed to satisfy this prerequisite and for this reason has been generally used in this work for the selective analysis of the phenolic compounds.

EXPERIMENTAL

Apparatus

The determinations were carried out with a Hewlett-Packard 1010 A chromatograph, modified with a Rheodyne 7120 sample injector and a 20-μl loop. A commercial reversed-phase column (250 × 4 mm). LiChrosorb RP-18, 10 μm, was employed for all separations. The eluting solvents were water (triply distilled)–methanol (LiChrosolv) mixtures. When the electrochemical detector (ElCD) was used an electrolyte (1 g/l $LiClO_4$ and 0.05 g/l H_2SO_4) was added to the eluting mixture.

A Metrohm 656 ElCD has a glassy carbon working electrode, a glassy carbon counter electrode and an Ag/AgCl reference electrode. A Metrohm VA 641 was employed as potentiostat and d.c. amplifier. If the sensitivity of the working electrode decreased, the surface was renewed by mechanical polishing using Al_2O_3 powder (0.3 μm).

Reagents

Phenolic standards and other chemicals were technical grade or better and used

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without further purification. The phenol standard solutions were acidified to increase their stability.

Preparation of standard fulvic and humic acid solutions

The isolation of fulvic and humic acids was carried out as described elsewhere[4].

To a 2.5-g sample of humus were added 50 ml of 0.1 *M* $Na_4P_2O_7$. The mixture was shaken for 1 h and then centrifuged for 5 min at 1500 *g*. An aliquot of solution was acidified with sulphuric acid to pH 2 and the precipitated humic acids were separated by centrifugation. The supernatant was evaporated to dryness thus giving the fulvic fraction.

The two fractions so obtained were used for the evaluation of possible interferences in the water analysis.

RESULTS AND DISCUSSION

HPLC of hydroxynaphthalenes

The analytical separation of monohydroxy- and dihydroxynaphthalenes by HPLC on a RP-18 column was achieved under the conditions reported in Table I, and typical chromatograms are shown in Figs. 1–3.

From Table I it can be seen that the separation of the twelve compounds is possible using two eluents of different polarities. With the more polar solvent, methanol–water (1:3.5), all the isomers with the two hydroxyl groups not on the same benzene ring are separated, whereas for the isomers with the two hydroxyl groups on the same ring a less polar eluent, methanol–water (1:1.3), was needed. Also, α- and β-naphthols can easily be separated with the latter solvent.

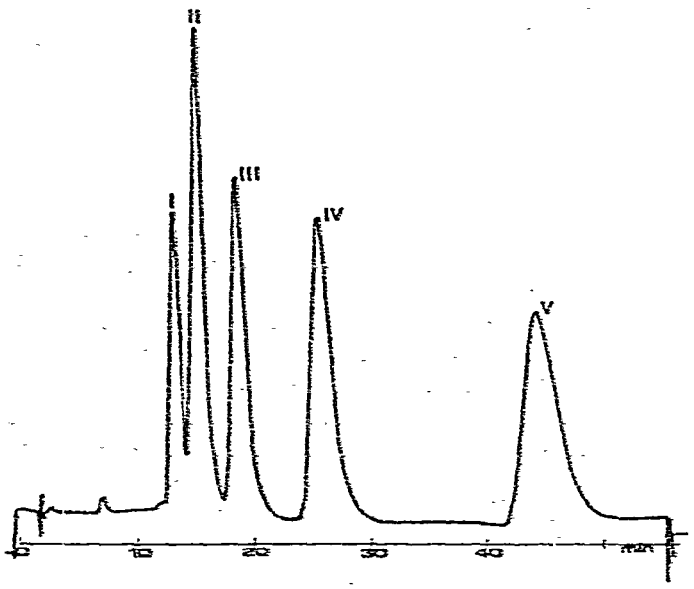

Fig. 1. Separation of hydroxynaphthalenes (group A). Column: RP-18. Eluent: methanol–water: (1:3.5). EICD applied potential: 1.20 V.

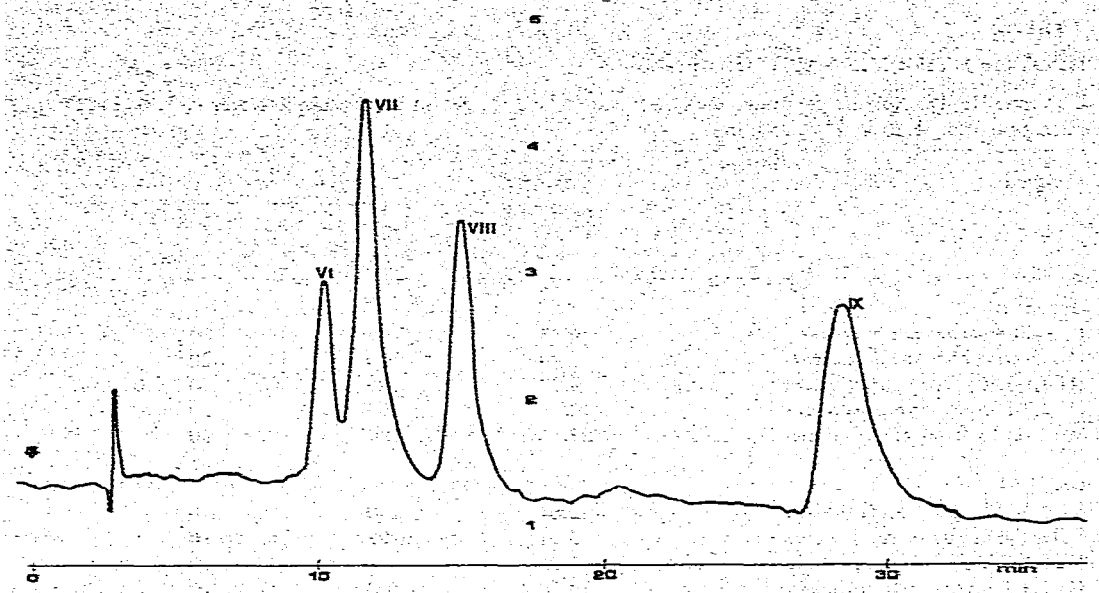

Fig. 2. Separation of hydroxynaphthalenes (group B). Column: RP-18. Eluent: methanol–water (1:1.3). UV detector: 254 nm.

The solute retention is a function of a reversible association between the apolar ligand bonded to the surface of silica particles and the apolar moiety of the solute molecules[5,6]. The elution order in group A follows the increasing length of the hydrocarbon aromatic chain. The 1,8-dihydroxy compound is eluted only with the more apolar solvent.

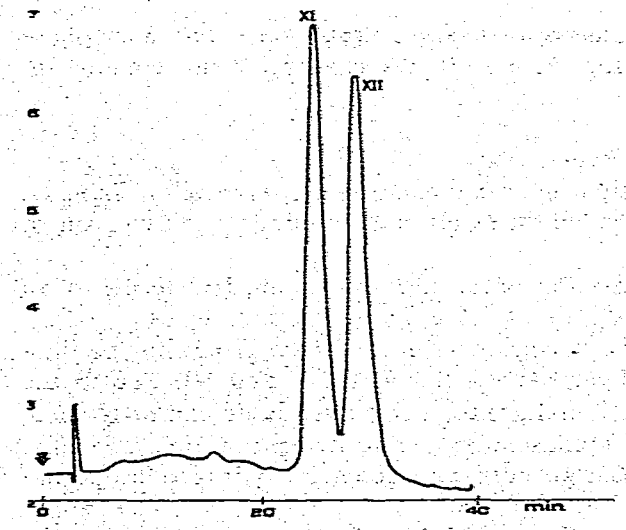

Fig. 3. Separation of α-, β-naphthols. Column: RP-18. Eluent: methanol–water (1:1.3). ElCD applied potential: 1.20 V.

TABLE I

HPLC OF HYDROXYNAPHTHALENES

Column: RP-18. Eluent: group A, methanol–water (1:3.5); group B, methanol–water (1:1.3). ElCD applied potential: 1.20 V. UV: 254 nm.

Compound	*Capacity factor, k'*	*Detection limits*	
		ElCD (ppb)	*UV (ppm)*
Group A			
I 2,6-Dihydroxynaphthalene	6.33	100	20
II 1,5-Dihydroxynaphthalene	7.44	100	20
III 1,6-Dihydroxynaphthalene	9.33	100	20
IV 2,7-Dihydroxynaphthalene	12.88	100	20
V 1,7-Dihydroxynaphthalene	23.66	100	20
Group B			
VI 1,3-Dihydroxynaphthalene	2.64	100	10
VII 2,3-Dihydroxynaphthalene	3.14	100	10
VIII 1,4-Dihydroxynaphthalene	4.35	—*	10
IX 1,8-Dihydroxynaphthalene	9.14	100	10
X 1,2-Dihydroxynaphthalene	17.14	—**	—**
XI β-Naphthol	7.89	100	10^2
XII α-Naphthol	9.29	100	10

* No response on ElCD.

** Impure compound.

When the two hydroxyl groups are present on the same ring the retention time increases dramatically and a change of eluent is necessary; so, in group B, the 1,3-, 2,3- and the 1,2-isomers follow the expected elution order, considering as above the length of the hydrocarbon chain.

In this picture of the interactions between the solute and the stationary phase the anomalously large value of k' for the 1,4-dihydroxynaphthalene remains unexplained.

HPLC of hydroxylated benzo- and alkylphenones

Chromatographic results for eight hydroxyphenones are reported in Table II. For the sake of simplicity we have divided the series of compounds into two groups: benzophenones and alkylphenones.

For the benzophenones, the elution order can be explained in terms of an approach similar to the one previously used for the hydroxynaphthalenes.

In compound I both the aromatic rings bear an hydroxyl group having little possibility of interaction with the hydrocarbon ligand. In V and VI, due to the position of the OH relative to the carbonyl group, a hydrogen bond can be formed, enhancing the hydrophobic effect with consequently longer retention times. Finally, VIII is eluted last because one of the OH groups is engaged in an hydrogen bridge and the other is methylated.

In the alkylphenones a further effect has to be taken in account, that is the length of the aliphatic chain bonded to the carbonyl group. The capacity factor is

TABLE II

HPLC OF HYDROXYLATED BENZO- AND ALKYLPHENONES

Column: RP-18. Eluent: methanol–water (1.5:1); flow-rate 1 ml/min. ElCD applied potential: 1.20 V.

Compound	*Capacity factor, k'*	*Detection limits*	
		ElCD (ppb)	*UV (ppm)*
I 4,4'-Dihydroxybenzophenone	0.40	100	1
II 2,4-Dihydroxyacetophenone	0.62	100	1
III 4-Hydroxypropiophenone	0.71	100	1
IV 2-Hydroxyacetophenone	1.66	100	1
V 2,4-Dihydroxybenzophenone	2.86	100	1
VI 2,2'-Dihydroxybenzophenone	3.24	100	1
VII 2-Hydroxypropiophenone	3.38	100	1
VIII 2-Hydroxy-4-methoxybenzophenone	9.95	100	1

dependent on the molecular surface for solutes of homologous series[7,8] and as a consequently the larger k' of VII compared to IV, is as expected.

Performance of electrochemical detector

HPLC-ElCD has been used successfully for picomole levels of readily oxidized

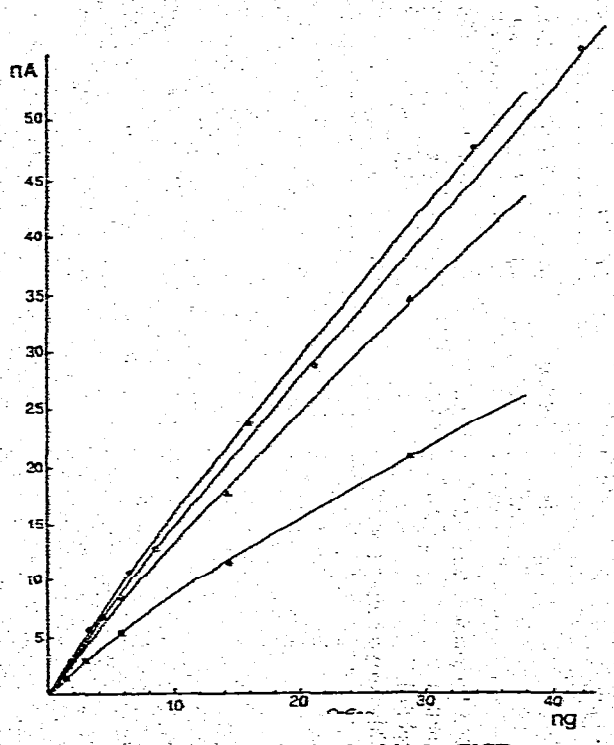

Fig. 4. Standard curves obtained with the ElCD at an applied potential of 1.20 V. ●, 1,3-Dihydroxynaphthalene; ▲, β-naphthol; ■, α-naphthol; ○, 4,4-dihydroxybenzophenone.

TABLE III

MEAN VALUE AND REPRODUCIBILITY OF PEAK HEIGHTS

The mean values and the standard deviations (R.S.D.s) were calculated over six measurements; nA denotes nanoamperes.

Compound	$5 \cdot 10^{-7}$ *M*		10^{-6} *M*		$2 \cdot 10^{-6}$ *M*		$5 \cdot 10^{-6}$ *M*		10^{-5} *M*	
	Av. nA	*R.S.D. (%)*	*Av. nA*	*R.S.D. (%)*	*Av. nA*	*R.S.D. (%)*	*Av. nA*	*R.S.D. (%)*	*Av. nA*	*R.S.D. (%)*
1,3-Dihydroxynaphthalene	2.8	3.6	5.5	4.4	10.3	1.4	22.9	2.7	46.1	0.5
β-Naphthol	2.6	7.7	4.7	8.4	8.2	2.4	17.0	2.8	33.2	1.7
α-Naphthol	1.7	9.1	2.8	8.5	5.3	4.5	10.6	3.1	20.3	2.9
4,4'-Dihydroxybenzophenone	3.2	2.9	6.6	3.3	12.2	4.1	27.4	2.4	54.2	1.7

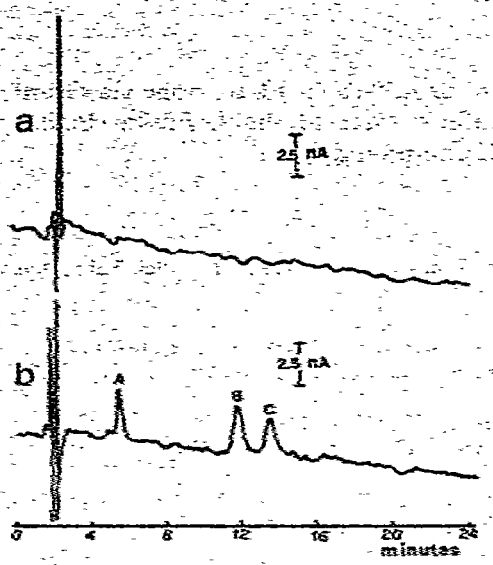

Fig. 5. Analysis of Savena water (a) and of Savena water fortified (b) with 104 ppb 1,3-dihydroxynaphthalene (A), 80 ppb β-naphthol (B) and 80 ppb α-naphthol (C). Sample size: 20 μL. Eluent: methanol–water (1:1). Column: RP-18. ElCD applied potential: 1.20 V.

species such as phenolic compounds[8,9]. We have evaluated the suitability of this selective detector for the analysis of hydroxylated polyaromatic compounds.

The detection limits (reported in Tables I and II) are of the order of 100 ppb, which allow the direct determination of trace amounts in water.

The ElCD has some drawbacks such as the extremely low response for 1,4-dihydroxynaphthalene and the rapid sensitivity loss upon repeated injections of relatively concentrated solutions ($>10^{-5}$ *M*). However, with very dilute solutions the sensitivity loss, due probably to film formation on the electrode surface following the strong adsorption of such aromatic compounds, is less pronounced. It is advisable, therefore, to check daily the detector sensitivity using a standard solution of low concentration (*e.g.*, 10^{-6} *M* phenol) and to regenerate the electrode when the decrease in sensitivity is $\geq 20\%$.

To determine the linearity of the detector response, various dilutions of four representative phenolic compounds were made and injected in the chromatograph. Table III gives the peak heights and the relative per cent standard deviations. Fig. 4 shows a plot of peak height *versus* the amount of sample injected. Although the curves show some non-linearity, it is not very pronounced.

We have evaluated the possibility of interferences with some constituents of the organic matter of soil dissolved in water, such as humic and fulvic acids. Two solutions containing respectively the fulvic and the humic fractions extracted as described in the Experimental section were chromatographed. Although the concentrations were quite high (*ca.* 100 mg/l), no peaks appeared in the chromatograms and no interferences with the determination of the hydroxy compounds were found.

An actual sample of water collected from the Savena river has been analysed together with a sample fortified with three representative compounds (Fig. 5). Also in this case there is no sign of interference from any compounds contained in the water.

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