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SEPARATION OF SUBSTITUTED PHENOLS, INCLUDING ELEVEN PRIORITY POLLUTANTS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

High-performance liquid chromatography separation by isocratic elution of a wide range of substituted phenols, including the so-called priority pollutants, is investigated. By careful selection of the eluting solvent mixture, the method may be adapted for the routine analysis of phenols particularly phenolic aqueous environmental samples.

INTRODUCTION

Environmental aspects of phenols and their substituted derivatives have become increasingly important in recent years. Procedures for their separation and determination include gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The spectrophotometric determination of phenols using the corresponding 4-aminoantipyrine derivatives is well-established[1,2], but the method is not applicable to many *para*-substituted phenols[3]. Further, it is not very satisfactory for the determination of phenols in which the substituent is an alkyl, aryl, nitro, benzoyl, nitroso or aldehyde group[3]. It is used, therefore, primarily for the determination of total phenol concentration, as it is unable to distinguish between different phenols. Many substituted phenols occur in the environment some of which are highly toxic even in small concentrations.

The United States Environmental Protection Agency (U.S.E.P.A.)[5] have listed eleven as priority pollutants. Of the methods reported using HPLC, some employed UV detection[6–9] while fluorescence detection was employed in others[10]. Further, the separation of large component mixtures was achieved only by gradient elution[6,11] and in some cases by temperature programming[12] and pH-control[13]. For precise, quantitative results, isocratic elution, in preference to gradient elution, has been recommended[14].

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The present paper reports the HPLC separation of a wide range of substituted phenols including the so-called priority pollutants by isocratic elution using UV detection. The method proposed for the separation is suitable for adaptation to routine analysis.

EXPERIMENTAL

Analyses were carried out with an LDC Constametric HPLC pump, utilizing a variable-wavelength UV detector, LDC spectromonitor (III) Model 1204. Radial compression HPLC was employed using the RCM 100, radial compression module, the column being a Radial Pak C_{18} cartridge, 5 μm, 10 cm × 8 mm, supplied by Waters Assoc. (Milford, MA, U.S.A.). Output from the detector was monitored by a Hewlett-Packard 3388 integrator microprocessor.

Samples were injected by a Waters Intelligent Sample Processor (W.I.S.P.) Model 71CB, which automatically and quantitatively transfers an operator-designated aliquot of sample to the fluid stream of the liquid chromatography system. In the present work, 20-μl injections were used throughout.

Solvents and reagents

Acetonitrile: Waters Assoc. liquid chromatography solvent with 190 nm UV cut off. Acetic acid: UNICHROM, specially-purified for HPLC from Ajax Chemicals. Methanol: Waters Assoc. liquid chromatography solvent. All solvents were degassed by passing them through a Millipore PTFE filter, 0.45 μm pore size, 47 mm diameter under vacuum.

Standards for the various phenols were prepared as 1000 mg/l stock solutions in acetonitrile and stored in a freezer. Dilutions were carried out daily, as required. All the phenols were of AR grade quality and obtained from Aldrich or Eastman Kodak.

RESULTS

Preliminary experiments were carried out to determine the optimal isocratic eluent mixtures for the separation of the different phenols. The following eluent mixtures were found to be the most satisfactory: (I) acetonitrile–water–acetic acid (50:50:0.1); (II) acetonitrile–water–acetic acid (37.5:62:5:0.1); (III) acetonitrile–water–methanol–acetic acid (20:55:25:0.1); (IV) acetonitrile–water–acetic acid (40:60:0.1).

A flow-rate of 2 ml/min was used throughout the work. Dual wavelength detection at 280 nm and 254 nm (0.05 a.u.f.s.) was employed with attenuation giving 160 μV/cm and 40 μV/cm, respectively. The phenols investigated and their capacity factors for the different eluent mixtures are shown in Table I. Table I also includes the ratio of wavelength absorptivities (280/254 nm) for each phenol. Those phenols denoted by an asterisk are the eleven priority phenols (phenol, *p*-nitrophenol, 2,4-dinitrophenol, *o*-chlorophenol, *o*-nitrophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 2-methyl-4,6-dinitrophenol, 2,4,6-trichlorophenol and pentachlorophenol).

Using eluent mixture I, chromatograms were obtained for a mixture of the 21

TABLE I

THE PHENOLS INVESTIGATED AND THEIR CAPACITY FACTORS FOR THE DIFFERENT ELUENT MIXTURES

Phenol	*Capacity factors (k') (280 nm)*				*Ratio 280 nm/ 254 nm*
	Eluent I	*Eluent II*	*Eluent III*	*Eluent IV*	
Resorcinol	0.32	0.33	0.35	0.34	1.45
Phenol*	1.07	1.45	1.48	1.28	1.42
p-Nitrophenol*	1.29	1.75	2.32	1.68	2.99
p-Cresol	1.57	2.09	2.69	2.05	5.78
2,4-Dinitrophenol*	1.77	2.44	3.06	2.39	0.70
o-Chlorophenol*	1.86	3.09	3.21	2.47	4.53
m-Chlorophenol	2.20	3.37	4.31	3.12	4.48
3,5-Dimethylphenol	2.38	3.54	4.50	3.32	3.95
o-Nitrophenol*	2.71	3.76	4.66	3.58	1.67
2,4-Dimethylphenol*	2.68	3.94	5.54	3.77	6.47
4-Chloro-3-methylphenol*	3.11	4.72	7.58	4.46	6.76
2,4-Dichlorophenol*	3.62	5.98	11.43	5.60	8.94
2-Methyl-4,6-dinitrophenol*	4.22	7.86	9.75	6.17	1.05
3,5-Dichlorophenol	4.95	8.63	16.16	7.84	9.03
2,3,6-Trichlorophenol	5.90	9.50	–	8.98	7.02
2,3,4-Trichlorophenol	5.84	10.18	–	9.35	2.62
2,4,6-Trichlorophenol*	6.85	13.88	–	10.90	5.00
2,4,5-Trichlorophenol	6.94	14.40	–	11.16	3.95
2,3,4,6-Tetrachlorophenol	11.10	–	–	16.21	1.36
2,3,5,6-Tetrachlorophenol	11.61	–	–	17.00	1.56
Pentachlorophenol*	18.75	–	–	24.44	0.32

* Priority pollutants.

phenols listed in Table I. It was found that: 2,4-dinitrophenol and *o*-chlorophenol, and *o*-nitrophenol and 2,4-dimethylphenol co-elute (Table II) and that the following pairs of trichlorophenols, 2,3,6- and 2,3,4-trichlorophenol, 2,4,6- and 2,4,5-trichlorophenol, are also unseparated, the remaining 13 phenols are separated, therefore, although several pairs of phenols are unresolved by the use of eluent mixture I, nevertheless, it offers a method by which the entire range of components present in a sample may be surveyed.

Using eluent mixtures II or IV, it was found that the separations could be refined and improved. It can be seen from Table I, that the trichlorophenols 2,3,6- and 2,3,4-trichlorophenol, and 2,4,6- and 2,4,5-trichlorophenol now separate. However, 2,4-dinitrophenol and *o*-chlorophenol are still unseparated.

By changing to eluent mixture III, separation of all priority pollutants, including, 2,4-dinitrophenol and *o*-chlorophenol is now achieved. The result for a mixture of phenol, *p*-nitrophenol, 2,4-dinitrophenol, *o*-chlorophenol, *o*-nitrophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol and 2-methyl-4,6-dinitrophenol can be seen in Fig. 1. Results for all priority pollutants using eluent mixtures I, II and III are included in Table II.

It is worth noting that 2,4,6-trichlorophenol and pentachlorophenol can be separated more rapidly from the other priority pollutants using eluent mixtures I or IV (Table I).

TABLE II

DETECTION LIMITS (ng PHENOL) AT 280 nm, DETERMINATIONS BASED ON PEAK HEIGHT

LDL = lowest detectable limit in nanogram; R = regression correlation coefficient; R.S.D. (%) = percent relative standard deviation where capacity factor calculated as average from 6 runs; m = slope; $y = mx + c$, correlation curve; k' = capacity factor of phenol in presence of mixtures as represented in Fig. 1 (Eluent III).

Phenol	*Eluent I*					*Eluent II*					*Eluent III*				
	LDL	*m*	*R.S.D. (%)*	*R*	*k'*	*LDL*	*m*	*R.S.D. (%)*	*R*	*k'*	*LDL*	*m*	*R.S.D. (%)*	*R*	*k'*
Phenol	2.50	1.333	0.25	0.9999	1.066	3.34	0.901	0.14	0.9996	1.454	3.58	1.194	0.19	0.9998	1.484
p-Nitrophenol	1.05	2.847	0.23	0.9998	1.295	1.30	2.312	0.16	0.9997	2.009	1.68	1.785	0.14	0.9996	2.333
2,4-Dinitrophenol	0.98	3.041	0.22	0.9998	–	1.73	1.735	0.18	0.9997	–	1.30	2.308	0.11	0.9999	3.066
o-Chlorophenol	2.71	1.106	0.20	0.9998	–	4.33	0.692	0.18	0.9999	–	3.77	0.796	0.08	0.9997	3.300
o-Nitrophenol	0.41	7.318	0.20	0.9999	–	1.40	2.145	0.11	0.9996	4.296	1.44	2.078	0.06	0.9999	5.805
2,4-Dimethylphenol	2.51	1.196	0.13	1.0000	–	3.73	0.804	0.11	0.9999	4.540	4.50	0.667	0.07	0.9999	5.861
4-Chloro-3-methylphenol	4.08	0.736	0.13	0.9997	3.066	6.04	0.497	0.09	0.9998	5.505	8.38	0.358	0.05	0.9997	7.593
2,4-Dichlorophenol	4.11	0.730	0.11	0.9999	3.738	7.32	0.411	0.06	0.9995	6.956	8.14	0.369	0.04	0.9999	9.952
2-Methyl-4,6-dinitrophenol	0.76	3.961	0.08	0.9994	4.295	1.32	2.273	0.04	0.9993	7.777	0.95	3.145	0.03	0.9992	8.429
2,4,6-Trichlorophenol	11.28	0.266	0.06	0.9996	6.959	38.02	0.789	0.02	0.9998	-					
Pentachlorophenol	21.32	0.141	0.06	1.0000	18.639										

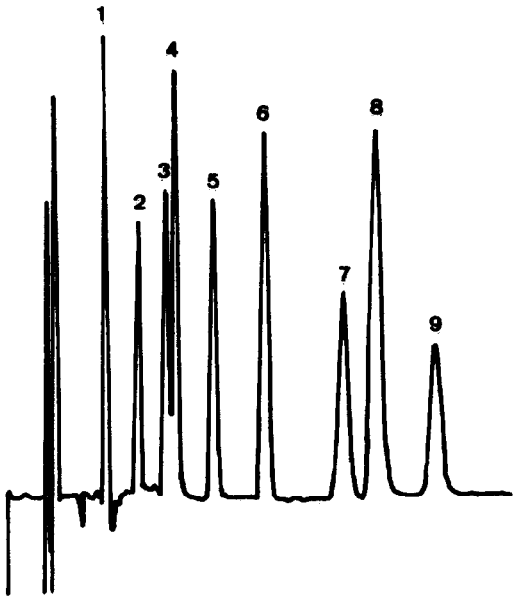

Fig. 1. Chromatogram of a phenol mixture at 280 nm. Eluent mixture III, chromatographic conditions as described in text. Peaks 1 = phenol; 2 = *p*-nitrophenol; 3 = 2,4-dinitrophenol; 4 = *o*-chlorophenol; 5 = *o*-nitrophenol; 6 = 2,4-dimethylphenol; 7 = 4-chloro-3-methylphenol; 8 = 2-methyl-4,6-dinitrophenol; 9 = 2,4-dichlorophenol.

It is, therefore, suggested that, as gradient elution involves many errors and a lengthy analysis time, the eleven priority pollutants might be separated by sequential isocratic elution, *e.g.*, elution of sample using eluent mixture I (25-min run time), followed by analysis, if desired, of sample using eluent mixture (3) (17-min run time). If, on the other hand, the separation of the trichlorophenols is of interest, eluent mixture II would be used.

Detection limits

Calibration graphs were drawn of peak height *vs.* quantity injected for each of the eluent mixtures I, II and III. Results are shown in Table II. For phenol, *o*-chlorophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol and 2,4-dichlorophenol the upper limit of sample was 80 ng for 2,4,6-trichlorophenol and pentachlorophenol 200 ng and for *p*-nitrophenol, 2,4-dinitrophenol, *o*-nitrophenol and 2-methyl-4,6-dinitrophenol 20 ng. The lower limits were as indicated in Table II, where the signal-to-noise ratio was at least 5:1. Further, in Table II, the slope m and regression coefficient R for each calibration $y = mx + c$ are given.

Various dilutions of each phenol could be run under identical conditions, as the noise level was extremely low. The lowest detectable limit was determined by the integrator capacity to integrate the peak arising from the injected sample. The precision of separation was determined by performing the separations six times and from the results, calculating the percent standard deviation (Table I).

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