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DETERMINATION OF PHENOLS BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL AND UV DETECTION

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An analytical method has been developed for the determination of 15 phenols in water, using HPLC and both electrochemical and UV detection. The elution was carried out with the mobile phase 1 % aqueous acetic acid-acetonitrile (66 : 34). The preconcentration was achieved by liquid-liquid extraction. Performance of the LC system was checked. Linearity of the detector response and detection limit without concentration were determined.

Introduction

Phenolic compounds occur widely in nature. They are building units of plants and are formed as products in metabolic processes. They serve as important raw materials in chemical industry. Many phenolic compounds are used for the preservation of wood and drugs [1].

However, phenols are of interest as environmental pollutants. Concentrations as low as a few μg/l affect the taste and odour of water and fish [2].

They are known to be widespread as components of industrial waste. Also, they are often present in alternate fuels such as oil shale and in the waste effluents of oil shale industry.

A great number of procedures have been developed for the quantitative analysis of phenols including spectroscopic techniques [3]

and gas chromatography [4]. Several studies have been done using HPLC on the trace phenols in aqueous samples [2, 5-8]. An extensive comparison of the separation of alkylphenols by HPLC was made by Schabron [9].

In this work the determination of alkylphenols and hydroxyphenols has been investigated.

The method of analysis of phenolics includes the clean-up, preconcentration and chromatographic steps. The preconcentration was achieved by liquid-liquid extraction with diethylether. The chromatographic separation of phenols was performed by using both UV and electrochemical detection. LC-ED was applied, employing an amperometric detector. ED and UV detection were compared in order to check the improvement in sensitivity and the limits of detection.

The following is a description of the HPLC method for the analysis of phenols in waste water.

Experimental

Materials

Phenol and *p*-cresol were obtained from Carlo Erba (Milan, Italy); 2,3-dimethylphenol, 2,5-dimethylphenol, 3,5-dimethylphenol, 5-methylresorcinol, 2,3,5-trimethylphenol, 2,3,6-trimethylphenol and 2,4,6-trimethylphenol were purchased from Aldrich (Milwaukee, WI, USA); resorcinol, *o*-cresol, 3,4-dimethylphenol and 2,6-dimethylphenol were obtained from Merck (Darmstadt, Germany) and *m*-cresol from Fluka (Buchs, Switzerland); hydroquinone was received from Scharlau (Barcelona, Spain).

Acetonitrile and methanol (HPLC-grade) were obtained from J.T. Baker (Deventer, Netherlands); chloroform, dichloromethane and acetic acid from Merck while diethylether from SDS (Peypin, France) was an analytical reagent grade.

Water was purified by using the Culligan system (Barcelona, Spain). All the solvents were passed through 0.45 μm nylon filter before use.

Apparatus

Experiments were performed by using a Hewlett-Packard (Palo Alto, CA, USA) Series 1050 LC with an isocratic pump, a column furnace and an automatic injector. The electrochemical amperometric detector was an HP 1049 A (Hewlett-Packard). A D-2500 Chromato-Integrator Merck-Hitachi integrator was used.

For UV studies, a Hewlett-Packard Series 1050 LC with a quarternary pump, a column furnace and UV detector HP 1050 VWD were used.

Chromatographic Conditions

Separation of the compounds was achieved by using an HP C_{18} Hypersil ODS (250 mm × 4 mm i.d., 5 µm) analytical column from Hewlett-Packard and a Pelliquard LC-18 (20 µm) precolumn (20 mm × 4 mm i.d.) from Supelco (Gland, Switzerland).

The elution was carried out at a flow rate of 1.000 ml/min with the mobile phase 1 % aqueous acetic acid - acetonitrile (66 : 34). The volume of sample injected by automatic injection was 20 µl. The separation was carried out at 30 °C.

The electrochemical detector worked in the amperometric mode. The optimum working potential was set at +1100 mV between the glassy carbon working electrode and Ag/AgCl reference electrode. The detector has a pre-treatment function to clean automatically the working electrode, used after every injection, cycling the applied potential two times between −600 mV and +1200 mV.

The wavelength of the UV detector was set at 280 nm.

Preparation of Standards and Samples

Stock standard solutions were prepared at 500 mg/l concentration in acetonitrile and kept in the dark at 4 °C. Dilutions of the stock standard solutions were made with mobile phase immediately before use.

Diethylether was chosen as the best solvent from a number of organic solvents investigated for the extraction of phenols from water samples.

A 100 ml portion of HPLC-water, spiked with standard phenols (15 ppm of each), was acidified with sulphuric acid (1 ml/l) to a pH of 2. The solution was then extracted with one 20 ml and three 10 ml portions of diethylether. For all the extractions the separating funnels were shaken for 10 min and two phases were allowed to separate for 10 min. The organic phases were collected and 2 ml of acetonitrile added to avoid losses by evaporation. Then the extract was evaporated to 2-3 ml in a water bath. The sample was redissolved in 50 ml of acetonitrile.

Results

Chromatographic Method

Several elution conditions were investigated. Satisfactory separation between 15 phenols was obtained by using the isocratic mobile phase 1 % acetic acid - acetonitrile (66 : 34). The background current of the electrochemical cell for this eluent was 50-70 nA. In this case the phenols were resolved in 21 min. Both EC and UV detection were used. The separation was better upon using UV detection. The problem was probably due to a longer connection tubing between the injector and the

column when using EC detection. Figures 1 and 2 show the chromatograms of the 15 phenols studied.

Different proportions of acetonitrile/acetic acid 1 % were tested. Retention times increased when lower amounts of acetonitrile were used, whereas 2,3,5-trimethylphenol and 2,3,6-trimethylphenol coeluted and so did 2,5- and 2,6-dimethylphenol .

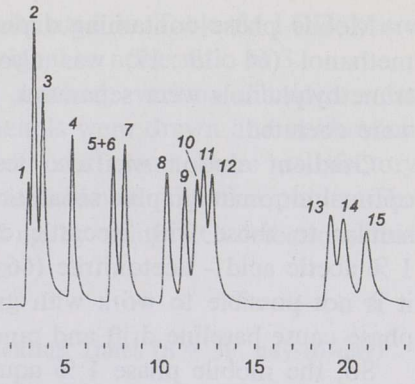

Fig. 1. LC-EC chromatograms of phenols. Analytical column: 250 × 4 mm i.d. HP C18 Hypersil ODS. Mobile phase: 1 % acetic acid - acetonitrile (66 : 34) at 1.0 ml/min. Electrochemical detection with applied potential 1.1 V. Peaks: (*1*) hydroquinone, (*2*) resorcinol, (*3*) 5-methylresorcinol, (*4*) phenol, (*5*) *m*-cresol, (*6*) *p*-cresol, (*7*) *o*-cresol, (*8*) 3,4-dimethylphenol, (*9*) 3,5-dimethylphenol, (*10*) 2,3-dimethylphenol, (*11*) 2,5-dimethylphenol, (*12*) 2,6-dimethylphenol, (*13*) 2,3,5-trimethylphenol, (*14*) 2,3,6-trimethylphenol, (*15*) 2,4,6-trimethylphenol. Concentration for each phenol 0.6 mg/l

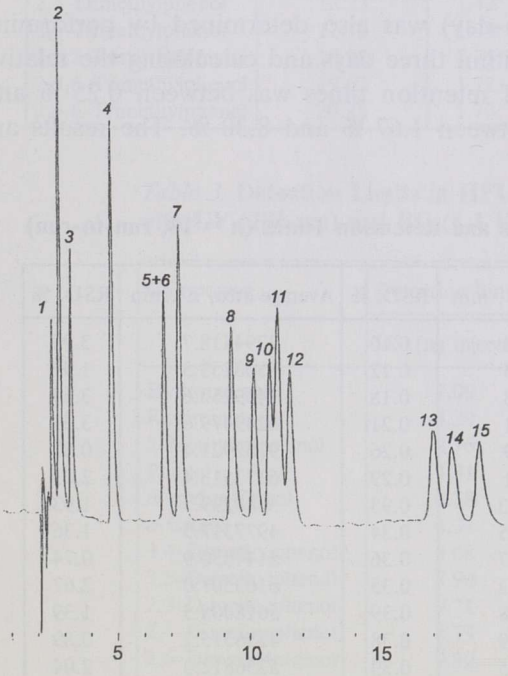

Fig. 2. LC-UV chromatograms of phenols. Analytical column: 250 × 4 mm i.d. HP C18 Hypersil ODS. Mobile phase: 1 % acetic acid - acetonitrile (66 : 34) at 1.0 ml/min. UV detection (at 280 nm). Peaks are the same as in Fig. 1. Concentration for each phenol 9 mg/l

To improve the separation, ternary mobile phases were tested. When using the mobile phase pH 4.3 acetate buffer - acetonitrile - methanol (65 : 25 : 10), the background current was 150-200 nA. The phenols were resolved in 28 min, but the separation was insufficient. 2,5- and 2,6-dimethylphenol and 2,3,5- and 2,3,6-trimethylphenol did not separate.

Mobile phase containing a phosphate buffer at pH 7 - acetonitrile - methanol (64 : 19 : 17) was also studied. Under these conditions all trimethylphenols were separated, but 2,3-, 3,5- and 2,6-dimethylphenols were coeluted.

Gradient elution was also tested with a UV detector to have the optimal chromatographic separation of phenols, however, the results were similar to those with isocratic elution by the using the mobile phase 1 % acetic acid - acetonitrile (66 : 34). With an electrochemical detector it is not possible to work with gradients because changes in the mobile phase cause baseline drift and random noise.

So, the mobile phase 1 % aqueous acetic acid - acetonitrile (66 : 34) was chosen to confirm the presence of phenols in real samples.

Precision

The repeatability (run-to-run) of the standard separation was determined by performing the separation for ten times and calculating the percentage of relative standard deviation for areas and retention times. The standard solution contained 1 ppm of each phenol. The relative standard deviation (RSD %) of peak areas was between 0.74 % and 3.93 %. The RSD % of retention times was between 0.10 % and 0.93 %.

The reproducibility (day-to-day) was also determined by performing the separation for ten times within three days and calculating the relative standard deviation. RSD % of retention times was between 0.25 % and 1.75 % and for peak areas between 1.67 % and 8.30 %. The results are tabulated in Tables 1 and 2.

Table 1. **Repeatability of Areas and Retention Times (n = 10, run-to-run)**

Compound	Average t_r, min	RSD, %	Average area, nA min	RSD, %
Hydroquinone	2.88	0.10	2294418.7	3.93
Resorcinol	3.15	0.12	8308253.3	1.92
5-Methylresorcinol	3.63	0.18	7469250.6	3.64
Phenol	5.21	0.21	6249479.6	3.51
m-and *p*-Cresol	7.39	0.26	9762921.4	0.87
o-Cresol	8.01	0.29	6177513.4	2.01
3,4-Dimethylphenol	10.13	0.93	5688299.5	1.93
3,5-Dimethylphenol	11.15	0.34	4977317.5	1.36
2,3-Dimethylphenol	11.87	0.36	5144030.9	0.74
2,5-Dimethylphenol	12.22	0.35	6163507.0	2.67
2,6-Dimethylphenol	12.68	0.39	5618008.5	1.39
2,3,5-Trimethylphenol	18.99	0.38	4256315.2	0.89
2,3,6-Trimethylphenol	19.65	0.39	5236812.0	2.04
2,4,6-Trimethylphenol	20.88	0.37	3307432.5	3.35

The limits of detection (LOD) for phenolics were obtained by using both UV and EC detectors. The fact that EC detection allowed to monitor as little an amount as 5 pg of phenol and resorcinol was

especially significant. The results are tabulated in Table 3. LODs were calculated as responses higher than the signal to noise ratio of 3.

To determine the linearity of the method, various dilutions were made. Calibration graphs for all the phenols were drawn and calibration data were calculated. The correlation coefficients were satisfactory ($R^2 \geq 0.99$) for all the phenols and were achieved with five points. The curve was linear between 4 ng and 17 ng by using EC detection, and 20 ng and 170 ng by using UV detection.

Table 2. **Reproducibility of Areas and Retention Times (n = 30, day-to-day)**

Compound	Average t_r, min	RSD, %	Average area, nA min	RSD, %
Hydroquinone	2.89	0.25	2457149.5	8.30
Resorcinol	3.15	0.34	8276629.4	1.67
5-Methylresorcinol	3.63	0.49	7374431.7	2.91
Phenol	5.20	0.69	6170551.5	5.39
m-and *p*-Cresol	7.38	1.04	9674435.0	1.89
o-Cresol	8.01	1.06	6229583.7	3.33
3,4-Dimethylphenol	10.08	1.37	5543480.3	3.02
3,5-Dimethylphenol	11.16	1.44	4978812.2	2.49
2,3-Dimethylphenol	11.83	1.34	5093591.0	2.09
2,5-Dimethylphenol	12.23	1.43	6122975.8	5.37
2,6-Dimethylphenol	12.72	1.39	4883254.1	4.21
2,3,5-Trimethylphenol	18.98	1.75	5683393.3	4.55
2,3,6-Trimethylphenol	19.63	1.72	5366925.1	4.12
2,4,6-Trimethylphenol	20.88	1.70	3358280.5	4.43

Table 3. **Detection Limits in HPLC-Grade Water with UV (280 nm) and EC (1.1 V) Detection**

Compound	Detection limits	
	UV (ng injected)	EC (pg injected)
Hydroquinone	7.09	173.25
Resorcinol	4.29	5.42
5-Methylresorcinol	8.76	21.90
Phenol	4.31	5.39
m-and *p*-Cresol	6.78	20.71
o-Cresol	4.34	21.68
3,4-Dimethylphenol	8.68	54.23
3,5-Dimethylphenol	8.90	44.52
2,3-Dimethylphenol	9.71	90.11
2,5-Dimethylphenol	8.79	43.99
2,6-Dimethylphenol	9.32	43.28
2,3,5-Trimethylphenol	13.8	45.94
2,3,6-Trimethylphenol	14.5	85.65
2,4,6-Trimethylphenol	12.5	83.29

The Method of Phenol Extraction from Waste Water

When an analytical procedure is applied to a mixture of pollutants in aqueous solution, it is of primary importance to set up efficient purification and preconcentration steps.

One of the aims of this work was to check the clean-up and recovery efficiency of the procedures based on liquid-liquid extraction. Solid-phase extraction procedure was not adopted because of difficulties in determining reliable breakthrough volumes for individual phenols.

Table 4. **Recovery Rates for LLE**

Compound	Dichloromethan		Diethylether		Chloroform	
	Average, %	RSD, %	Average, %	RSD, %	Average, %	RSD, %
Hydroquinone	15.74	16.39	-	-	54.08	7.64
Resorcinol	-	-	106.09	3.87	10.47	7.82
5-Methylresorcinol	8.19	23.09	69.14	5.61	8.22	11.29
Phenol	73.21	2.90	94.36	1.67	72.07	3.26
m-and *p*-Cresol	91.19	2.19	95.64	1.04	91.25	1.65
o-Cresol	93.89	3.78	91.66	2.14	89.09	7.20
3,4-Dimethylphenol	95.22	4.27	87.08	2.67	100.47	7.46
3,5-Dimethylphenol	94.37	0.97	95.48	0.97	99.27	1.09
2,3-Dimethylphenol	94.35	1.50	97.90	6.12	104.52	4.22
2,5-Dimethylphenol	91.51	6.40	94.39	1.34	98.65	6.66
2,6-Dimethylphenol	98.73	4.78	103.40	4.62	91.12	1.04
2,3,5-Trimethylphenol	95.94	6.06	94.13	2.82	81.46	1.85
2,4,6-Trimethylphenol	76.76	6.06	88.22	3.23	79.82	1.63
2,3,6-Trimethylphenol	92.23	9.91	92.38	2.37	87.51	2.84

For the extraction of phenols from water sample, three solvents were investigated: dichloromethane, chloroform and diethylether. These three solvents were chosen because they are popular extraction solvents for the analysis of trace organics.

For the extraction with these solvents, a certain amount of approximately 1.5 mg of each phenol studied was added to 100 ml of HPLC-grade water. The water sample was adjusted to pH 2 with sulphuric acid (1 ml/l), and one 20 ml and three 10 ml portions of the organic phase were extracted.

To determine recoveries for the individual phenols, the areas obtained from the extracted water samples were compared to the areas of the standards. The recovery values were relatively high, but the best results were achieved by using diethylether, probably due to the hydrogen bonds between phenol and ether. To measure the accuracy and precision of the extraction method, three extractions of 100 ml volumes of spiked HPLC-grade water were performed. For each of these extractions the individual phenol peak areas were determined, and the percentage of relative standard deviation was calculated. Recoveries are tabulated (Table 4).

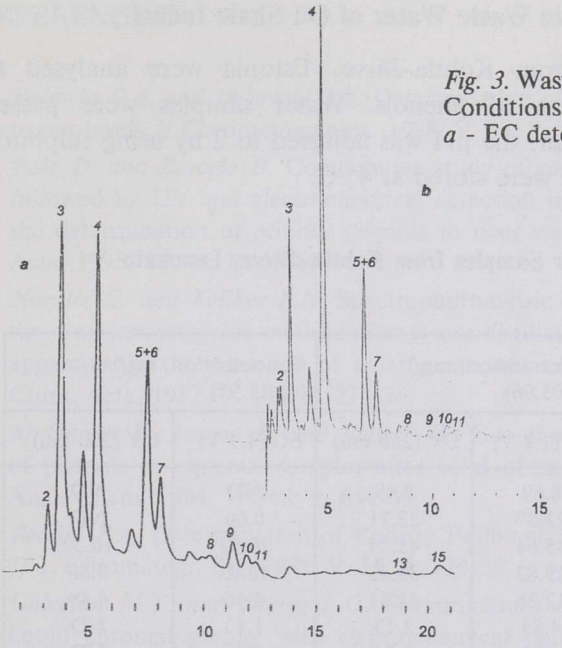

Fig. 3. Waste water sample (17.05.96). Conditions are the same as in Fig. 1. *a* - EC detection, *b* - UV detection

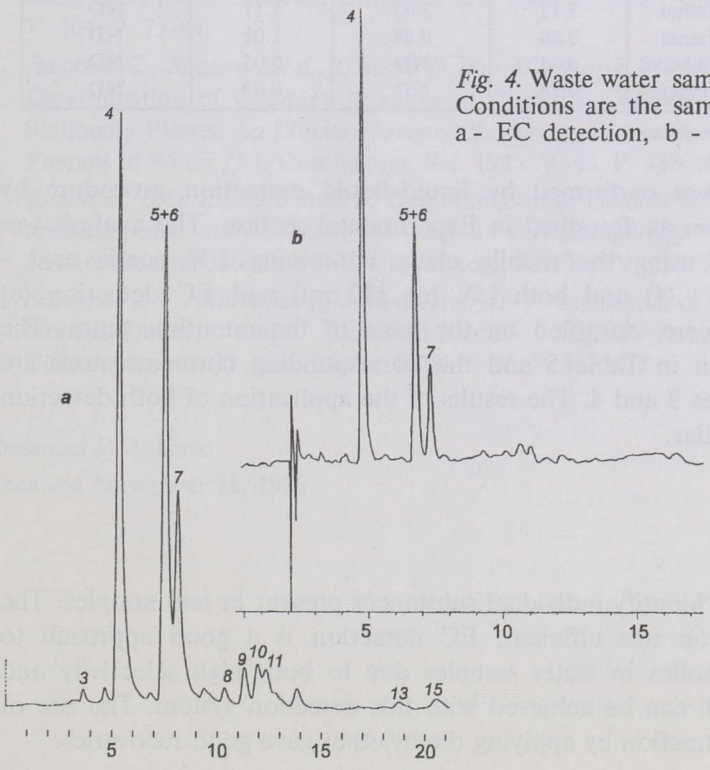

Fig. 4. Waste water sample (01.11.95). Conditions are the same as in Fig. 1. a - EC detection, b - UV detection

Determination of Phenols in Waste Water of Oil Shale Industry

Water samples coming from Kohtla-Järve, Estonia were analysed to measure the concentration of phenols. Water samples were passed through 0.45 μm nylon filter, the pH was adjusted to 2 by using sulphuric acid (1 ml/l), and samples were stored at 4 °C.

Table 5. **Analysis of Water Samples from Kohtla-Järve, Leachate from Ash Dump**

Compound	Concentration, mg/l (17.05.96)		Concentration, mg/l (01.11.95)	
	EC (1.1 V)	UV (280 nm)	EC (1.1 V)	UV (280 nm)
Resorcinol	8.69	8.49	ND	ND
5-Methylresorcinol	23.87	23.71	0.66	ND
Phenol	45.64	45.15	10.92	10.30
m-and *p*-Cresol	29.83	30.42	10.03	8.88
o-Cresol	12.35	11.81	4.46	4.49
3,4-Dimethylphenol	4.84	2.13	1.13	ND
3,5-Dimethylphenol	4.99	2.83	0.99	ND
2,3-Dimethylphenol	3.11	2.43	1.11	ND
2,5-Dimethylphenol	0.26	0.84	1.08	ND
2,3,5-Trimethylphenol	0.43	ND	0.05	ND
2,4,6-Trimethylphenol	0.78	ND	0.03	ND

N o t e : ND - not detected.

Extraction was performed by liquid-liquid extraction procedure by using diethylether as described in Experimental section. The analysis was carried out by using the mobile phase containing 1 % acetic acid - acetonitrile (66 : 34) and both UV (at 280 nm) and EC detection (at 1.1 V). Peaks were identified on the basis of the retention times. The results are given in Table 5 and the corresponding chromatograms are shown in Figures 3 and 4. The results of the application of both detection systems are similar.

Conclusion

It is possible to identify individual substances present in real samples. The HPLC separation was efficient. EC detection is a good approach to determine phenolics in water samples due to both high selectivity and sensitivity which can be achieved with this detection system. The use of liquid-liquid extraction by applying diethylether gave good recoveries.

REFERENCES

1. *Baldwin D.A. and Debowski J.K.* Determination of phenols by HPLC in down to ppt levels // Chromatographia. 1988. V. 26. P. 186-90.
2. Pu*ig D. and Barcelo D.* Comparative study of on-line solid phase extraction followed by UV and electrochemical detection in liquid chromatography for the determination of priority phenols in river water samples // Anal. Chim. Acta. 1995. V. 311. P. 63-69.
3. *Norwitz G. and Keliher P.N.* Spectrophotometric determination of phenol by the 4-aminoantipyrine method after steam distillation in a semimicro Kjeldahl apparatus in the presence of a large amount of sodium chloride // Anal. Chim. Acta. 1982. V. 144. P. 273-76.
4. *Mußmann P., Levsen K. and Radeck W.* Gas-chromatographic determination of phenols in aqueous samples after solid phase extraction // Fresenius J. Anal. Chem. 1994. V. 348. P. 654-59.
5. *Realini P.A.* Determination of Priority Pollutant Phenols in Water by HPLC // J. Chromatogr. Sci. 1981. V. 19. P. 124-29.
6. *Galceran M.T. and Jauregui O.* Determination of phenols in sea water by liquid chromatography with electrochemical detection after enrichment by using solid-phase extration cartridges and disks // Anal. Chim. Acta. 1995. V. 304. P. 75-84.
7. *Baiocchi C., Roggero M. A., Giacosa D. and Marengo E.* HPLC Separation and Determination of Complex Mixtures of Phenolic Compounds on Different Stationary Phases. An Efficient General Screening Method for the Presence of Phenols in Water // J. Chromatogr. Sci. 1995. V. 33. P. 338-46.
8. *Ruana J., Urbe I. and Borrull F.* Determination of phenols at the ng/l level in drinking and river waters by liquid chromatography with UV and electrochemical detection // J. Chromatogr. A. 1993. V. 655. P. 217-26.
9. *Schabron J. F., Hurtubise R. J. and Silver H. F.* Separation of Alkylphenols by Normal-Phase and Reversed-Phase High-Performance Liquid Chromatography // Anal. Chem. 1978. V. 50. P. 1911-17.

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