Journal of Chromatography, 257 (1983) 69–79
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,444

DETERMINATION OF PHENOLS IN WATER SAMPLES AS 4-AMINOANTIPYRINE DERIVATIVES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received October 12th, 1982)

SUMMARY

The determination of 22 monohydric phenols by high-performance liquid chromatography after derivatization with 4-aminoantipyrine is described. The reaction variables were evaluated in order to examine the performance of the method at low ppb* concentrations. The chromatographic behaviour of the derivatives was investigated by carrying out separations on different bonded-phase columns, which provided a satisfactory selectivity under isocratic elution conditions. Separations of complex mixture obtained on normal and reversed bonded phases, in addition to bathochromic and hypsochromic effects shown by derivatives, provided effective information for identifying individual phenols. The technique was applied to the determination of phenols at ppm and ppb concentrations in water and wastewater samples.

INTRODUCTION

Phenols occur as organic pollutants in many industrial effluents and as a result of the environmental transformation of both natural and synthetic chemicals[1,2]. The determination of individual phenols is very important because some of them, at low parts per billion (ppb) concentrations, cause undesirable organoleptic properties in drinking water upon chlorination[3].

Examples of trace determinations of phenols by gas chromatography and liquid chromatography have been reported[4–9]. Unfortunately, their direct determination in actual samples by high-performance liquid chromatography (HPLC) with a UV detector often suffers from poor detector sensitivity and the effects of interfering compounds. Hence sample derivatization has been used to overcome these problems, especially with liquid chromatographic techniques[10,11].

Determinations of phenols by HPLC after derivatization with 3-methyl-2-benzothiazolinone hydrazone[12] and *p*-nitrobenzene diazonium tetrafluoroborate[13]

* Throughout this article, the American billion (10^9) is meant.

0021-9673/83/0000–0000/$03.00

have been reported. However, these derivatization agents do not have any particular advantages because other compounds, as well as phenols, give azo dyes. The oxidative coupling reaction with 4-aminoantipyrine (4-AAP), performed in the presence of an alkaline oxidizing agent, however, is specific to phenols[14] and the colorimetric test with 4-AAP is the most widely used standard method for phenols[15,16]. The advantages, limitations and interferences of this method have been widely studied and are well known[17–20]. The only drawback is that certain *para*-substituted phenols do not react with 4-AAP.

Separations of phenol as 4-aminoantipyrine quinoneimine derivatives have not yet been carried out by HPLC. This paper describes a study of the potential of coupling the specificity of this derivatization reaction with the sensitivity and separation power of HPLC for 22 monohydric phenols. In particular, we evaluated the reaction variables, taking into account the coupling of derivatization with 4-AAP with chromatographic separation of the dye product at low ppb levels, and investigated the chromatographic behaviour of the phenol derivatives in different HPLC systems.

The application of the technique to the determination of phenols at ppm and ppb concentrations in industrial wastewaters and water samples is discussed.

EXPERIMENTAL

The analyses were performed with a Spectra Physics Model 3500B liquid chromatograph equipped with a Model 770 spectrophotometric detector at wavelengths variable from 200 to 600 nm, a rotary valve injector with a 10-μl standard sample loop and a Leeds and Northrup Speedomax XL681A recorder.

The columns (300 × 3.9 mm I.D.), pre-packed with μBondapak CN, μBondapak C_{18} or μBondapak Phenyl, were obtained from Waters Assoc. (Milford, MA, U.S.A.) and used at room temperature.

Chemicals

Phenol standards (98% pure) were obtained from Supelchem (Milan, Italy), kit no. 27-4-4570, and used in methanol solution. HPLC-grade solvents (Merck, Darmstadt, G.F.R.) were pre-filtered through a Millipore type F-H 0.5-μm filter and degassed. The reagents used were 4-AAP (Carlo Erba, Milan, Italy) as a 1.5% solution in distilled water, potassium hexacyanoferrate(III) (Carlo Erba) as a 2% solution in distilled water and Britton–Robinson[21] universal buffer (pH 8–9; μ = 0.09).

Procedure

To 100 ml of a water sample containing phenols (10–800 ppb) were added 10 ml of Britton–Robinson buffer, 1 ml of 4-AAP solution, 5 ml of potassium hexacyanoferrate(III) solution and 10 ml of chloroform. Before derivatization, samples were treated with a sufficient amount of EDTA disodium salt to prevent precipitation of calcium and magnesium salts when Britton–Robinson buffer was added. The reaction mixture was stirred for 10 min. The chloroform extract, separated and dried over filter-paper, was then subjected to HPLC. The separation of 4-AAP quinoneimine dyes was effected on the following chromatographic systems: (1) μBondapak C_{18} reversed phase with methanol–water as the mobile phase; (2) μBondapak

Phenyl reversed phase with methanol–water; (3) μBondapak CN reversed phase with methanol–water; and (4) μBondapak CN normal phase with *n*-hexane–tetrahydrofuran (THF). Various compositions of the mobile phases were tested. The flow-rate was kept at 1 ml/min. The red-orange derivatives were detected by their characteristic absorption in the visible region.

RESULTS AND DISCUSSION

Chromatographic study of derivatization reaction variables

The aim of this study was to examine the performance of the derivatization reaction at ppb concentrations. The optimum pH range for the oxidative coupling reaction was found to be 8–9. At. pH values higher than 9, the test for nitrophenols failed and also the chromatographic responses (peak height) for other phenols was lower. At pH values lower than 8, there were interferences from 4-AAP reaction by-products and aromatic amines[17,22] in the chromatographic separation.

The optimal amounts of reagents [4-AAP and potassium hexacyanoferrate(III)] were established according to Svobodova and Gasparič[19]. At the concentrations employed, these amounts were found to give complete reactions in 10 min for all of the phenols considered.

The reaction and extraction yields at ppb concentrations were measured for the phenol with respect to the pure solid derivative synthesized according to Svobodova and Gasparič[19]. Both the reaction and the extraction yields were quantitative under the conditions reported here.

Wavelength selection

Phenol derivatives absorb in the UV region and show maximum absorption in the range 420–520 nm. In the visible region these maxima are slightly different and also depend on solvent polarity[19].

These points were considered in order to optimize the detection conditions. A large number of reaction by-products of 4-AAP interfere in the chromatographic separation when UV detection is used, and the detection wavelength was therefore selected in the visible region where this effect does not disturb the separation.

The behaviour of the derivatives on μBondapak Phenyl with methanol–water (60:40) as the mobile phase is shown in Table I, where sensitivities relative to the phenol are reported at detection wavelengths of 460, 480 and 500 nm.

In the analysis of complex mixtures, the detector was adjusted to 480 nm, which is a compromise to achieve the highest sensitivity with methanol–water as the mobile phase. With *n*-hexane–THF as the mobile phase, the detection wavelength was optimized at 445 nm.

Relative sensitivities were calculated in the 10–800 ppb range using the peak height. A detailed examination of the sensitivities (Table I) shows that there are distinct hypsochromic and bathochromic effects correlated with the kind of substituent on the phenol ring, so additional identification information can be obtained by recording a separation at different wavelengths and measuring the bathochromic and hypsochromic effects.

TABLE I

DETECTOR SENSITIVITY AT DIFFERENT WAVELENGTHS FOR PHENOL DERIVATIVES IN THE 10–800 ppb CONCENTRATION RANGE

Detector sensitivity relative to phenol at 480 nm = 100%; 10–800 ppb represents concentration of phenols in aqueous samples before derivatization.

Compound	*Sensitivity (%)*		
	460 nm	*480 nm*	*500 nm*
Phenol	91.8	100	66.9
2-Methylphenol	67.9	62.2	50.7
3-Methylphenol	61.9	60.4	–
2-Ethylphenol	74.1	78.9	73.5
3-Ethylphenol	78.2	78.2	75.5
2,3-Dimethylphenol	30.1	30.3	25.2
2,5-Dimethylphenol	32.8	31.8	24.1
2,6-Dimethylphenol	32.6	28.5	26.2
3,5-Dimethylphenol	7.50	10.9	17.2
2,3,5-Trimethylphenol	7.00	9.30	11.3
2,3,6-Trimethylphenol	14.0	13.3	11.7
2,3,5,6-Tetramethylphenol	1.00	1.30	1.50
2-Chlorophenol	70.1	71.7	76.9
3-Chlorophenol	58.3	59.6	58.9
4-Chlorophenol	48.3	49.0	40.4
4-Chloro-3-methylphenol	26.9	28.5	34.7
2,3-Dichlorophenol	32.7	42.7	47.0
2,4-Dichlorophenol	21.3	32.6	41.1
2,6-Dichlorophenol	20.5	32.9	43.5
2,4,6-Trichlorophenol	32.2	28.8	24.6
2-Nitrophenol	1.33	1.95	2.52
3-Nitrophenol	1.10	1.27	1.37

Chromatography of 4-AAP quinoneimine derivatives

Table II reports the capacity factors, k', for the phenol derivatives in the different chromatographic systems numbered as described under Experimental.

In reversed-phase systems, separations were carried out using 50% and 60% methanol in water as the mobile phase. Increasing the percentage of methanol in the eluent decreases the k' values. The polar "nitrile" column does not yield separations in the reversed-phase mode, but gives more satisfactory results in the normal-phase mode. Systems 1a, 2b and 4 (Table II) provide good selectivity with adequate separation times using isocratic elution conditions. 4-AAP couples with phenols in the *para*-position and when it couples with phenols that carry a *para*-substituent such as a halogen, antipyrine dyes are formed by elimination of the *para*-substituent[23]. Thus phenol and 4-chlorophenol give the same antipyrine dye and hence show identical k' values (Table II); this similarly applies to 2-chlorophenol and 2,4-dichlorophenol, 2,6-dichlorophenol and 2,4,6-trichlorophenol and 3-methylphenol and 4-chloro-3-methylphenol.

On comparing the normal- and reversed-phase systems, an opposite order of elution of compounds is observed (see systems 4 and 1 and 2 in Table II). In the normal-phase mode the more highly substituted phenol derivatives are only slightly

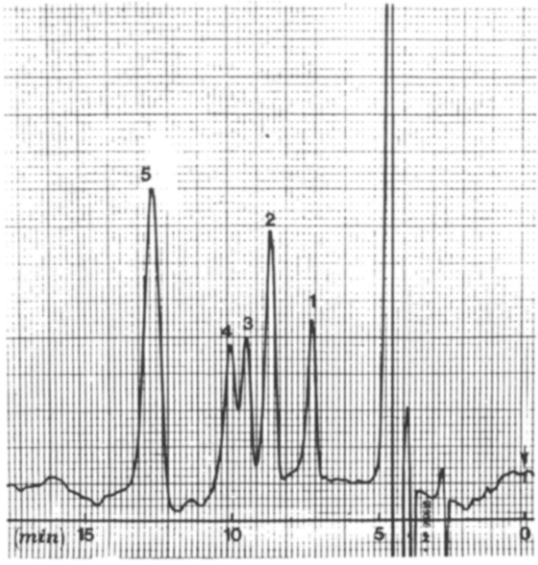

Fig. 1. Reversed-phase separation of alkylphenol derivatives. Conditions: column, μBondapak Phenyl (300 × 3.9 mm I.D.); mobile phase, methanol–water (60:40); flow-rate, 1 ml/min; detector, visible, 480 nm, 0.02 a.u.f.s.; sample volume, 100 ml. Peaks: 1 = 3,5-dimethylphenol, 520 ppb; 2 = 2,3-dimethylphenol, 110 ppb; 3 = 2,5-dimethylphenol, 130 ppb; 4 = 2,6-dimethylphenol, 130 ppb; 5 = 2,3,6-trimethylphenol, 335 ppb.

retained and 2-nitrophenol and phenol derivatives are strongly retained, whereas in the reversed-phase mode the same compounds show the strongest and weakest retention, respectively.

The peak asymmetry[10], A, observed on the C_{18} and Phenyl bonded columns was satisfactory (maximum 1.5) for all of the phenol derivatives. In contrast, strong asymmetry ($A \approx 2.3$) was observed for phenol and all of the non-*ortho*-substituted phenols (except 3-nitrophenol and 3-chlorophenol) on the CN bonded column in the normal-phase mode. Peak asymmetries on the CN column were found to be satisfactorily reduced by slightly modifying the *n*-hexane–THF mobile phase by adding 1 % of acetonitrile. These asymmetries seem not to depend on k' but probably on complex solute–stationary phase interactions. A more detailed consideration of this aspect is beyond the scope of this work.

We can conclude that the reversed-phase μBondapak C_{18} or Phenyl columns and the normal-phase μBondapak CN column (especially with acetonitrile in the mobile phase) are complementary for the separation and identification of complex phenol mixtures. Obviously the conditions for the chromatographic analysis of specific samples can be improved by varying the composition of the eluent mixture or even better by using gradient elution. The suggested compositions of the mobile phase provide satisfactory selectivity under isocratic elution conditions. The chromatogram in Fig. 1 shows the separation of alkylphenol derivatives on the μBondapak Phenyl column.

Detectability, linearity and interferences

The μBondapak Phenyl column with methanol–water (60:40) as the mobile

TABLE II

CAPACITY FACTORS, k', FOR PHENOL DERIVATIVES IN DIFFERENT CHROMATOGRAPHIC SYSTEMS

Flow-rate: 1 ml/min.

Compound	*Capacity factor, k'*					
	(1) μBondapak C_{18}		*(2) μBondapak Phenyl*		*(3) μBondapak CN/ Methanol–water (60:40)*	*(4) μBondapak CN/ n-Hexane–THF (60:40)*
	(a) Methanol–water (50:50)	*(b) Methanol–water (60:40)*	*(a) Methanol–water (50:50)*	*(b) Methanol–water (60:40)*		
Phenol	0.52	0.20	3.11	1.36	0.081	6.83
2-Methylphenol	0.79	0.24	3.93	1.79	0.064	1.78
3-Methylphenol	0.86	0.24	4.58	1.88	0.068	5.17
2-Ethylphenol	1.68	0.40	6.21	2.45	–	1.32
3-Ethylphenol	1.19	0.33	6.89	3.07	–	4.33
2,3-Dimethylphenol	2.14	0.51	–	2.43	–	1.68
2,5-Dimethylphenol	1.79	0.44	8.95	2.85	–	1.33
2,6-Dimethylphenol	1.82	0.37	–	3.03	–	0.87
3,5-Dimethylphenol	0.76	0.21	–	2.00	0.081	3.63
2,3,5-Trimethylphenol	1.70	0.35	8.75	2.60	–	1.53
2,3,6-Trimethylphenol	3.06	0.58	–	3.92	–	0.72
2,3,5,6-Tetramethylphenol	2.71	0.55	–	3.72	–	0.85
2-Chlorophenol	1.07	0.27	6.53	2.14	0.161	2.15
3-Chlorophenol	1.09	0.32	–	3.07	0.129	2.37
4-Chlorophenol	0.52	0.20	3.11	1.36	0.081	6.83
4-Chloro-3-methylphenol	0.86	0.24	4.58	1.88	0.068	5.17
2,3-Dichlorophenol	–	–	–	3.71	–	1.60
2,4-Dichlorophenol	1.07	0.27	6.53	2.14	0.161	2.15
2,6-Dichlorophenol	1.83	0.50	–	4.04	0.258	1.75
2,4,6-Trichlorophenol	1.83	0.50	–	4.04	0.258	1.75
2-Nitrophenol	1.00	0.33	3.91	1.63	–	7.18
3-Nitrophenol	0.92	0.28	5.49	2.13	–	2.34

phase was chosen for the investigation of detectability, linearity and aromatic amine interferences.

At 480 nm under the specified chromatographic conditions, the detection limit, as the amount injected that gives a peak height equivalent to twice the noise level at 0.01 a.u.f.s., is calculated to be 2 ng for phenol. For other phenols, the approximate detection limit can be determined by using the relative sensitivities reported in Table I.

The detection limit for phenol, in terms of concentration in a water sample analysed in accordance with the described experimental conditions (100-ml water sample, 10 ml of extraction solvent, injected volume 10 μl), was 20 μg/l. In trace analyses, the amounts of chloroform and water sample can be modified in order to improve the sensitivity. A concentration of 2 μg/l represented the detection limit for phenol when a 500-ml water sample was extracted with 5 ml of chloroform and a 10-μl volume of extract was injected. This concentration (2 μg/l) is that proposed as an "acceptable limit" for drinking water[24].

Following the procedure described under Experimental and the chromatographic conditions already specified, calibration graphs of peak height (h) *versus* concentration of standard sample (c) were determined for some derivatives. The results are shown in Table III, where the slope, α, of the calibration graphs ($h = \alpha c$), the correlation coefficient, the linear range and the error range of analysis, calculated according to Youmans[25], are reported for phenol, 2-methylphenol, 3-methylphenol, 2-chlorophenol and 3-chlorophenol.

The compounds that give the greatest interferences in phenol determinations using derivatization with 4-AAP are aromatic amines. The interference was studied with aniline as an example. Table IV lists the capacity factors for the 4-AAP derivatives of both phenol and aniline, together with the relative sensitivities under the

TABLE III

CALIBRATION FOR PHENOL DERIVATIVES

Chromatographic conditions: column, μBondapak Phenyl; mobile phase, methanol–water (60:40); detection, 480 nm (0.04 a.u.f.s.).

Compound	*Slope*, $\alpha \pm$ std. dev.****	*No. of runs*	*Correlation coefficient**, ϱ*	*Linear range investigated** (ppb)*	*Error range of analysis** (ppb $\pm$ std. dev.***)*
Phenol	0.023 ± 0.004	13	0.999	30–1000	100 ± 7 500 ± 6 1000 ± 13
2-Methylphenol	0.014 ± 0.003	5	0.996	60–500	100 ± 10 500 ± 47
3-Methylphenol	0.014 ± 0.003	5	0.996	40–500	100 ± 16 500 ± 43
2-Chlorophenol	0.016 ± 0.003	5	0.990	40–500	100 ± 19 500 ± 50
3-Chlorophenol	0.014 ± 0.003	5	0.998	40–500	100 ± 11 500 ± 27

* Calibration graphs: peak height (h,cm) *vs.* sample concentration (c,ppb).

** Statistics calculated according to Youmans[21].

*** Standard deviation at the 95% confidence limit.

TABLE IV

ANILINE INTERFERENCE

For chromatographic conditions, see Table III.

Compound	*Capacity factor, k'*	*Relative sensitivity*
Phenol	1.36	100
Aniline	1.35	0.21

chromatographic conditions specified in Table III. As the k' values of the phenol and aniline derivatives are similar, only the pH of the reaction mixture and not the selectivity of the chromatographic system is able to eliminate the interference due to aniline. The results in Table IV show that the aniline interference is virtually negligible when it occurs in the sample at the same concentration as the phenol.

Application and performance

The described technique permits one to determine 10 ppb of phenol in a 500-ml sample, extracted with 5 ml of chloroform. Fig. 2 shows the chromatogram for this analysis.

A specific example of the use of a wavelength shift for the identification of phenols with an uncertain assignment is shown in Fig. 3. A sample derivatized with 4-AAP was detected at three different wavelengths: 460, 480 and 500 nm. Peak 1 is easily

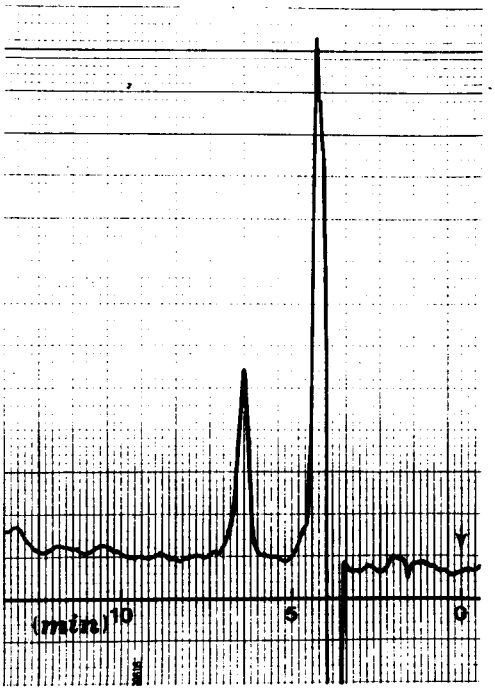

Fig. 2. Trace analysis: determination of phenol (10 ppb) in a 500-ml water sample. Conditions as in Fig. 1.

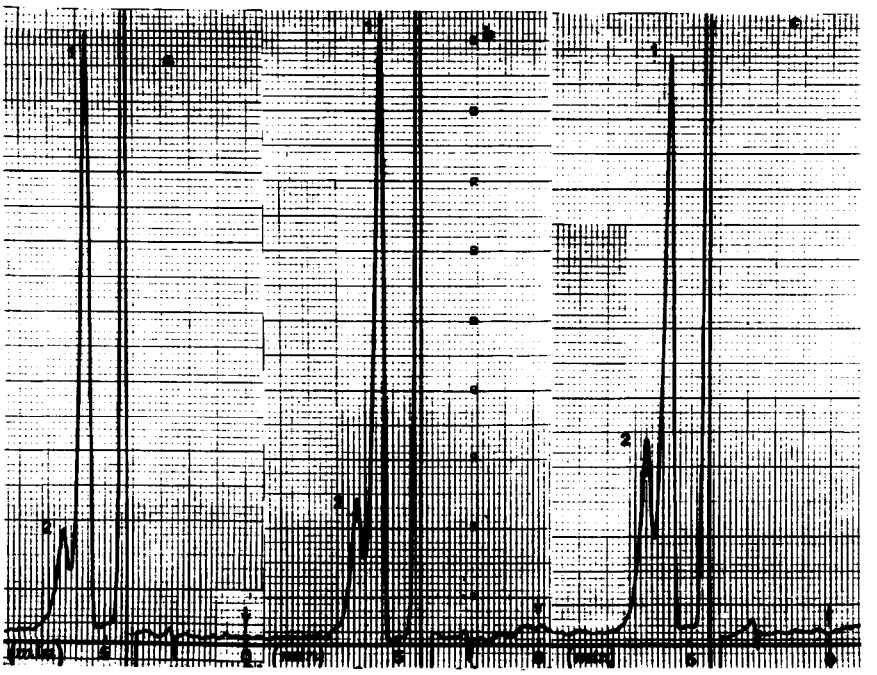

Fig. 3. Use of the bathochromic shift for identification purposes using a 100-ml water sample. Conditions as in Fig. 1, except detector, visible, 0.04 a.u.f.s. Peaks: 1 = phenol, 156 ppb; 2 = 2-nitrophenol, 1.8 ppm.

TABLE V

PHENOL DETERMINATION AND RECOVERY FROM ACTUAL WATER SAMPLES

For chromatographic conditions, see Table III.

Compound	*Sample*	*Concentration (ppb ± std. dev.*)*	*Amount added (ppb)*	*Amount found (ppb ± std. dev.*)*	*Difference found (%)*
Phenol	Wastewater from an industrial plant for phenolic resin production	166 ± 21	100	251 ± 10	−6
			200	383 ± 15	+5
			400	579 ± 20	+2
Phenol	Wastewater from wood vaporization	139 ± 16	100	225 ± 20	−6
			200	336 ± 30	−1
			400	561 ± 50	+5
2-Methylphenol	Wastewater from wood vaporization	145 ± 16	130	260 ± 10	−5
			270	415 ± 15	–

* Standard deviation at the 95% confidence limit.

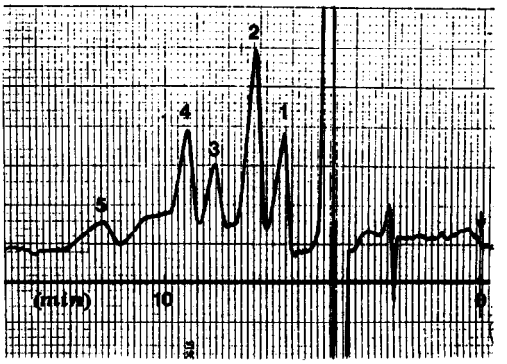

Fig. 4. Trace analysis: determination of chlorophenols in a 500-ml water sample. Conditions as in Fig. 1. Peaks: 1 = 4-chlorophenol, 1.0 ppb; 2 = 4-AAP product; 3 = 2,4-dichlorophenol, 1.0 ppb; 4 = 3-chlorophenol, 1.5 ppb; 5 = 2,4,6-trichlorophenol, 1.5 ppb.

distinguished as phenol and peak 2 can be identified as 2-nitrophenol rather than 2-methylphenol by its bathochromic shift.

Industrial wastewater samples in which phenols were present at about the 100 ppb level were analysed and linearity was investigated for routine determinations. The samples were spiked with different concentrations of phenol and 2-methylphenol. Table V reports the results for two wastewater analyses; the linearity range is satisfactory, and the determinations were carried out rapidly.

As 4-AAP derivatives have satisfactory thermal stability[19], a chloroform extract can be concentrated in a Kuderna–Danish concentrator equipped with a Snyder column and then analysed by HPLC.

Chlorophenols can be determined at 1–5 ppb concentration levels. Fig. 4 shows a chromatogram obtained from a 500-ml water sample extracted with 10 ml of chloroform. A 4-ml portion of extract was concentrated to 200 μl and analysed. Under these severe conditions, a 4-AAP reaction by-product was also separated and detected (peak 2 in Fig. 4).

Calibration graphs of detector response (peak area) *versus* amount of sample

TABLE VI

RECOVERY OF CHLOROPHENOLS IN TAP WATER FROM THE PUBLIC WATER SUPPLY OF FERRARA

For chromatographic conditions, see Table III.

Compounds tested	*Amount added (ppb)*	*Amount found (ppb)**
4-Chlorophenol	2.5	2.6
2,4-Dichlorophenol	2.0	1.9
3-Chlorophenol	3.0	3.1
2,4,6-Trichlorophenol	3.0	2.9

* Repeatability: ±12–20%.

injected were straight lines between 10 and 80 ng. Recovery tests, reported in Table VI, were made on chlorophenols in tap water from the public water supply of Ferrara City.

CONCLUSIONS

The coupling of 4-AAP derivatization with HPLC separation is a useful method for the analysis of individual phenols in mixtures and is rapid and very sensitive. The chromatographic conditions and amounts of chloroform and water sample can be modified in order to achieve a specific separation with the desired sensitivity. The bonded-phase column used provides considerable selectivity. Moreover, bathochromic and hypsochromic effects can be used for identification purposes. The suggested technique can therefore be applied to polluted water samples for the determination of phenols at ppm and ppb concentrations.

ACKNOWLEDGEMENTS

Industrial wastewater samples were supplied by Professor A. Cavallaro (Laboratorio Igiene Profilassi, Milan, Italy), to whom the authors are grateful.

This work was supported by the National Research Council of Italy (CNR), Programma Finalizzato "Promozione della Qualità dell'Ambiente —Sottoprogetto Acqua— IRSA", Contribution No. 80.01716.

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