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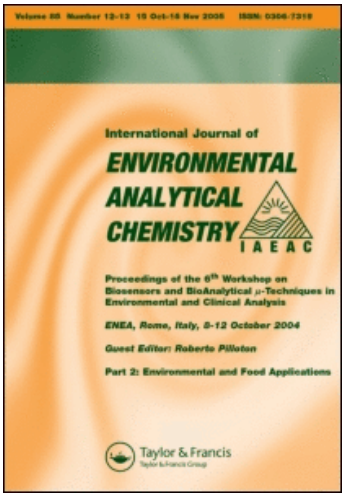

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M. W. F. Nielen[a]; J. De Jong[a]; R. W. Frei[a]; U. A.Th. Brinkman[a]
[a] Department of Analytical Chemistry, Free University, Amsterdam, the Netherlands

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Trace-Level Determination of Phenol by Liquid Chromatography with On-Line Precolumn Technology and Fluorescence Detection†

M. W. F. NIELEN, J. DE JONG, R. W. FREI and U. A. TH. BRINKMAN

Department of Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam, the Netherlands

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A strongly basic anion-exchange resin is used for the trace enrichment and automated sample handling of phenol, with subsequent determination by reversed-phase liquid chromatography with fluorescence detection. Because of the presence of high concentrations of ionic compounds in the water samples tested, phenol is first trapped on a relatively long precolumn filled with a highly hydrophobic packing material; during this step, (in) organic anions which are not retained, are flushed to waste. In the next step, phenol is desorbed from this column at high pH and sorbed in a small zone ("peak compression") on a short precolumn containing the anion exchanger.

In the analysis of tap and river water samples, the detection limit was found to be 10 ppt ($1:10^{11}$).

INTRODUCTION

Phenol and many of its chlorinated derivatives are present in (surface) water as a result of their frequent use in industrial activities and, in addition, because they are degradation products of several pesticides. In recent years, a number of chromatographic methods

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has been developed for the determination of chlorophenols in water samples. Buisson *et al.*[1] determined chlorinated phenols in aqueous samples by capillary gas chromatography with electron capture detection. Nair *et al.*[2] developed an isocratic liquid chromatographic (LC) system to separate 13 phenols on a reversed-phase column, and Shoup and Mayer[3] applied LC with electrochemical detection. Chriswell *et al.*[4] used an off-line preconcentration method using a $6 \times 1/2$ inch I.D. column packed with a resin-based anion exchanger. Phenols were sorbed at pH 12.5 and eluted with 4 M HCl, followed by liquid–liquid extraction, evaporation and, finally, gas chromatography. On-line trace enrichment and selective detection of chlorinated phenols was described by Werkhoven-Goewie *et al.*[5] who used a small precolumn packed with a styrene-divinylbenzene copolymer for preconcentration, a C18 separation column and a photochemical reaction detector in which the chlorinated phenols were dechlorinated on-line to obtain the highly fluorescent phenol. In this way (sub-) ppb levels could be selectively determined.

Many of the methods described so far do not include phenol itself and, if they do, the recovery is often low and/or the detection limit is rather high. In other words, there obviously still is a need for a—preferably automatable—LC method for the selective determination of sub-ppb levels of phenol in real samples, i.e., in the presence of ionic and non-polar contaminants. In this paper we describe a procedure in which phenol is, first, preconcentrated on a relatively long precolumn packed with a hydrophobic resin and—after the removal of inorganic compounds—transferred on-line to a second, small precolumn packed with a strongly basic anion exchanger. The LC separation is done on a C18 analytical column with subsequent fluorescence detection.

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EXPERIMENTAL

Apparatus

A Kontron (Zürich, Switzerland) LC system consisting of two Model 410 pumps, a pulse dampener, a MCS 670 Tracer valve switching unit and a Model 200 programmer was used; the set-up is shown in Figure 1. An LS-4 (Perkin-Elmer, Norwalk, CT, U.S.A.) fluorescence detector equipped with a 3 μl flow-cell was used for detection; the

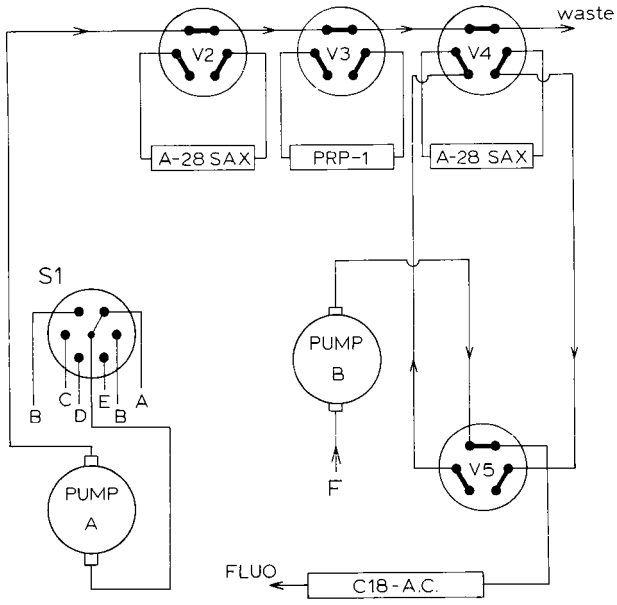

FIGURE 1 Experimental set-up for the on-line trace enrichment of phenol from water samples. S, low-pressure solvent selection valve; V, high-pressure switching valve. A, sample; B, water; C, sodium hydroxide (pH 11.5); D, 0.2 M sodium acetate containing 30% methanol; E, methanol–water (1:1); F, 0.4 M acetate buffer (pH 5.0)–methanol (1:1). Precolumns: 8 × 4.6 mm I.D. (Aminex A-28 at V_2), 20 × 4.6 mm I.D. (PRP_1 at V_3) and 10 × 3.0 mm I.D. (Aminex A-28 at V_4). Analytical column, 10 cm × 3.0 mm I.D. packed with CP-Spher C18.

excitation and emission wavelength were 271 and 297 nm, respectively. Chomatograms were analog recorded on a W+W 900 (Kontron) recorder and processed manually.

Stationary phases and columns

Trace enrichment was carried out on a 20 × 4.6 mm I.D. home-made stainless-steel precolumn,[6] packed with PRP_1 (cf. below), and on a slightly modified 10 × 3.0 mm I.D. Chrompack (Middelburg, the Netherlands) preconcentration column. The precolumns were hand-packed[6] by using a syringe filled with a slurry of either the spherical 10 μm styrene-divinylbenzene copolymer PRP_1 (Hamilton, Reno, NV, U.S.A.) in methanol or the 11 μm resin-based Aminex A-28

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(Bio-Rad, Richmond, CA, U.S.A.) quaternary ammonium anion exchanger in an aqueous buffer.

The analytical column was a 100×3.0 mm I.D. glass cartridge prepacked with 8 μm CP-Spher C18 (Chrompack).

Chemicals

HPLC-grade methanol and water, analytical grade acetic acid, sodium acetate, sodium hydroxide, carbonate-free hydroxide "Dilut-it" and phenol were obtained from J. T. Baker (Deventer, the Netherlands). 4-Chlorophenol was obtained from Fluka (Buchs, Switzerland). Sodium hydroxide solutions were sealed with a calcium chloride cap to prevent carbon dioxide uptake by these solutions.

All eluents were degassed in an ultrasonic bath under vacuum.

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Procedures

Stock solutions of phenol were prepared by weighing and dissolving in methanol, and stored at -20°C. The solutions were diluted with HPLC-grade water to obtain standard solutions at the (sub) ppb level. Breakthrough curves of phenol on the various precolumns were recorded according to the procedure reported in ref. 7, using 2–200 ppb standard solutions and a flow-rate of 2 ml min^{-1}.

Tapwater samples were analyzed without any pretreatment. The surface and waste water samples were filtered over a 0.8 μm membrane filter, in the latter case after 40–1,000-fold dilution with HPLC-grade water.

The automated sample handling and trace enrichment and the subsequent on-line elution of the sorbed phenol were performed using the experimental set-up given in Figure 1. Five ml samples are introduced via pump A onto the PRP_1 precolumn (at V_3, cf. Figure 1); phenol and many other organic compounds are retained but the inorganics, which would interfere in the trace-enrichment step on the anion-exchanger precolumn, are flushed to waste. Next, the PRP_1 precolumn is flushed with 3 ml of HPLC-grade water to ensure that all inorganics are flushed out. One now switches pump A to sodium hydroxide (pH 11.5), which, in order to remove systemic phenol impurities, first passes through an 8×4.6 mm I.D. anion-exchange precolumn (at V_2, cf. Figure 1) and then the PRP_1 precolumn.

Phenol is eluted from this PRP_1 precolumn in its anionic form and the phenolate ion is then sorbed on a small anion-exchanger precolumn (at V_4, cf. Figure 1) where peak compression occurs. Finally this precolumn is eluted via pump B, with a mobile phase consisting of 0.4 M acetate buffer (pH 5.0)–methanol (1:1), to the C18 separation column and phenol is detected in its neutral form.

The PRP_1 precolumn is regenerated during the analytical separation via pump A with methanol–water (1:1). The anion-exchange precolumn is regenerated by the mobile phase during the elution step. The other anion-exchange precolumn, used for reagent purification, is regenerated every five analyses via pump A with a 0.2 M sodium acetate solution (pH 7.0) containing 30% methanol. Finally the PRP_1 and both Aminex A-28 precolumns are flushed with water before the next sample is introduced. The entire procedure is summarized in Table I; details about the switching program can be found in the Appendix.

TABLE I

General procedure using the set-up of Figure 1.[a]

1. Trace enrichment of phenol on PRP_1
2. Flushing PRP_1 with water
3. Transfer of phenol to Aminex A-28 at pH 11.5
4. Flushing Aminex A-28 with sodium hydroxide (pH 11.5)
5. Forward-flush desorption from the anion-exchanger to the C18 analytical column
6. Regeneration of PRP_1 with methanol–water (1:1)
7. Flushing precolumns with water

[a]The time-based switching program is given in the Appendix.

RESULTS AND DISCUSSION

Retention of phenol on different precolumns

From earlier studies and preliminary experiments it is known that trace enrichment of phenol (in its neutral form) from 10 ml aqueous samples on styrene-divinylbenzene and other similar polymer sorbents is only possible when using relatively long precolumns of several centimeters. However, under these conditions many other organic compounds—both of a polar and non-polar nature—will

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also be retained and, consequently, clean-up will be insufficient. Trace enrichment of phenol ($pK_a = 9.9$) in its anionic form can be carried out on an anion-exchange resin. However, the breakthrough volume will decrease rapidly if inorganic anions are present in the sample matrix and phenol dissolved in, e.g., saline is not retained at all.

By combining both types of packing materials mentioned above, one should be able to solve the various problems. As regards the trace enrichment of phenol on PRP_1, precolumn loading has to be done from an acidic or, preferably, a neutral solution. For example, on a 20×4.6 mm I.D. column, the breakthrough volume at pH 7 is already more than 20 ml. On the other hand, the breakthrough volume rapidly drops to about 1 ml or less for pH values higher than the pK_a of phenol. If phenol is eluted in its anionic form without using a modifier, i.e., by a steep pH increase only, many other organic compounds will remain on the polymeric resin. Phenol itself can be reconcentrated on a second anion-exchanger-containing precolumn from which it may be eluted in its neutral form onto a reversed-phase separation column. The neutral condition is optimal for the highly sensitive and selective fluorescence detection of the analyte. The proposed procedure effects an efficient removal of polar and non-polar (in)organic interferences; besides, the relatively broad phenol profile obtained on the PRP_1 precolumn will be compressed on the anion-exchanger in the second stage.

We preferred to use an aqueous alkaline solution of pH 11.5 instead of pH 12.0 for the transfer of phenol from the PRP_1 to the Aminex A-28 precolumn, becuase of the much lower breakthrough volume on the anion exchanger observed at pH 12.0 (6 vs. 20 ml), due to a stronger competition between hydroxyl and phenolate ions. Trace enrichment on a precolumn of the same dimensions (10×3.0 mm I.D.) but packed with a 40 μm silica-based instead of the 11 μm resin-based anion-exchanger was not successful: breakthrough occurred immediately.

Elution of phenol from the anion exchanger

Initially, desorption of phenol from the precolumn packed with Aminex A-28 was attempted with an aqueous solution containing 0.1 M sodium perchlorate and 0.01 M perchloric acid. This procedure

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was not successful. Although the perchlorate acted as a displacer, phenol was found to be only partly converted into its neutral form by this solution, probably as a result of the large excess of hydroxyl ions released. In addition, regeneration of the precolumn became rather complicated. A mobile phase consisting of 1.0 M sodium acetate buffer (pH 5.0)–methanol (1:1) was found to provide an efficient elution of phenol in its neutral form. However, due to the high salt concentration, inner filter effects occurred during detection and the fluorescence yield was seriously decreased. Further work revealed that the best compromise between signal enhancement and efficiency of the elution procedure was obtained with a 0.4 M sodium acetate buffer of pH 5.0–methanol (1:1). In addition, with this mobile phase, regeneration of the precolumn was found to be superfluous.

Forward-flush elution was preferable to backflush elution, the latter showing a negative influence on band broadening due to the tendency to form a gap at the top of the precolumn. This is contrary to our earlier observations with cation-exchange resins.[8]

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Purity of reagents

Sodium hydroxide pellets usually contain about 1% of sodium carbonate which, being a bivalent anion, will compete successfully with the phenolate anion. It can be calculated that 50 ml of 0.1 M sodium hydroxide (which was used in the preliminary experiments for precolumn regeneration) will be able to convert the anion-exchange precolumn completely into the carbonate form after which regeneration will be rather difficult. It is therefore essential to use carbonate-free sodium hydroxide solutions, which are commercially available under the tradename "Dilut-it". In addition, all solutions should be protected against atmospheric carbon dioxide as outlined in the experimental section.

Unfortunately, the carbonate-free sodium hydroxide solutions are sold in plastic containers, which were shown also to contain traces of phenol. This problem was solved by inserting a relatively large anion-exchange precolumn (8 × 4.6 mm I.D.) for on-line eluent purification during the phenol transfer (with sodium hydroxide pH 11.5) from the PRP_1 to the small anion-exchange precolumn.

General performance

Table II summarizes the analytical data obtained by the final procedure using the set-up of Figure 1 and the general procedure of Table I. It can be seen that traces of phenol can be successfully determined with good recovery. This is especially true when we keep in mind that phenol is preconcentrated and eluted twice and, in addition, the phenolate ions have to compete with hydroxyl ions which are present in 10^6-fold excess (as compared to 0.3 ppb anionic phenol) when phenol is reconcentrated on the anion-exchange precolumn!

TABLE II

Analytical data for the automated determination of phenol. Conditions: 5 ml sample solutions analyzed according to the procedure of Table I (cf. Figure 1); data based on peak area measurements

Criterion	Level	Result
Repeatability	0.3 ppb ($n=10$)	$\pm 2.4\%$
Recovery	0.4 ppb 4.0 ppb	89.5% 86.7%
Linearity (r)	0.03–100 ppb ($n=7$)	0.9994
Detection limit	S/N = 3/1	50 pg or 10 ppt

Application to real samples

Tap water (Free University) was analyzed and found to contain less than 0.01 ppb phenol. River water (River Waal, Lobith, the Netherlands) contained approximately 0.1 ppb phenol. Figure 2 shows chromatograms of river water, tap water and HPLC-grade water, respectively, spiked with 0.4 ppb of phenol. The selectivity of the method is clearly demonstrated at this trace level. The repeatability of the method in analyzing river water samples was found to be $\pm 4\%$ RSD ($n=5$) at the 0.5 ppb level. The recovery of spiked river water as compared to spiked HPLC-grade water was $101 \pm 4\%$ ($n=3$).

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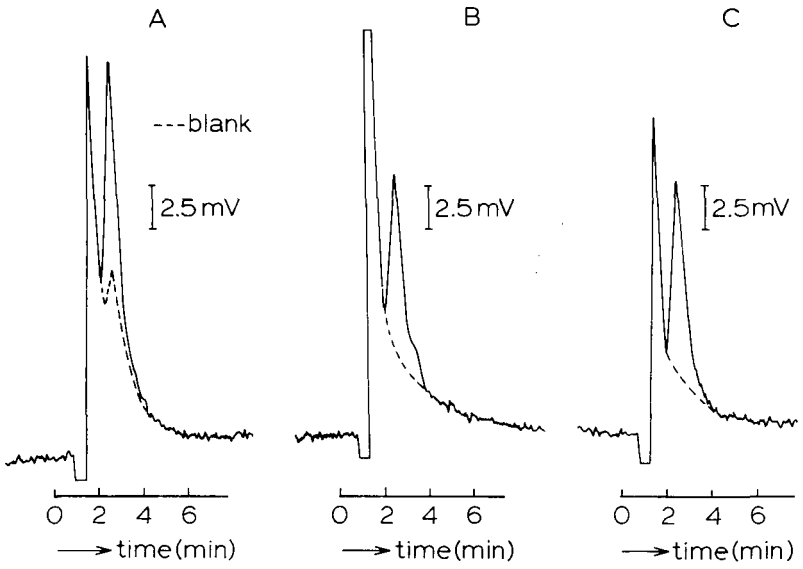

FIGURE 2 Chromatograms of 5 ml water samples without added phenol (-----) and spiked with 0.4 ppb phenol (———), analyzed using the set-up of Figure 1 and the general procedure of Table I. (A) Waal river water; (B) tap water; (C) HPLC-grade water standard solution; Analytical column, 10 cm × 3.0 mm I.D. CP-Spher C18. Eluent, 0.4 M acetate buffer (pH 5.0)–methanol (1:1) at 0.4 ml min^{-1}. Detection by fluorescence at 271 nm excitation and 297 nm emission wavelength. Other conditions as in Figure 1.

We also analyzed several industrial waste water samples for phenol and found concentrations of between 60 ppb and 6 ppm. Blank urine (after hydrolysis with sulphuric acid to release phenol from its conjugates) contained 1 ppm phenol, which is a well-known metabolite of tyrosine. However, one should realize that for such high levels other, less expensive methods, are available. The present method should be considered as a procedure for the selective determination of real trace levels in aqueous samples.

The excellent selectivity of the proposed method is demonstrated by comparing Figures 2 and 3. The latter one represents the preconcentration of phenol from 5 ml water samples from two different rivers on the non-selective PRP_1 precolumn with an additional flush step of 6 ml HPLC-grade water. The anion-exchange precolumns were not used in this case and the phenol was simply

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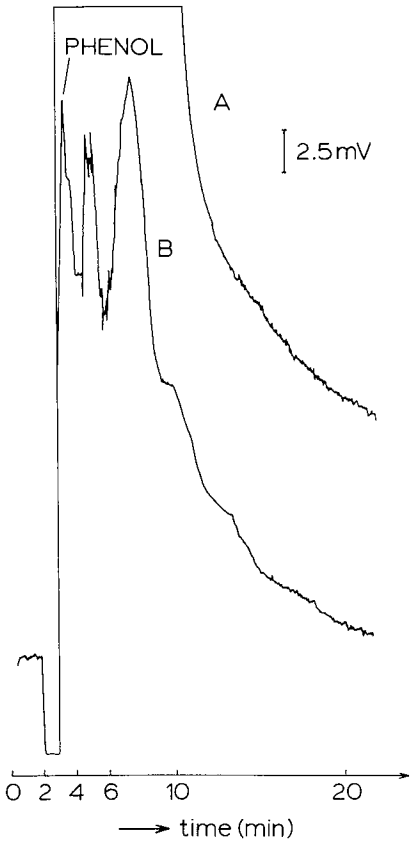

FIGURE 3 Chromatograms of 5 ml river water samples spiked with 0.4 ppb of phenol obtained after trace enrichment on a 20 × 4.6 mm precolumn packed with PRP_1, a flush with 6 ml of water and direct elution to the analytical column with methanol–water (1:1). Other conditions as in Figure 2. Samples taken from the rivers Amstel (A) and Waal (B).

eluted with 50% methanol onto the C18 separation column. We can see that the combined use of fluorescence detection and an anion-exchange precolumn combined with PRP_1 results in such an increase in selectivity, that trace-level determination of phenol in real samples is easily attainable.

To demonstrate the selectivity of the present method for phenol over other—e.g., lower chlorinated—phenols, we analyzed a sample

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spiked with a 100-fold excess of 4-chlorophenol. No peak due to the latter compound was observed.

CONCLUSIONS

Trace levels of phenol in water samples can be selectively determined by liquid chromatography with on-line precolumn trace enrichment and clean-up, and fluorescence detection. The combination of a relatively large non-selective precolumn, packed with PRP_1, and a small precolumn packed with selective material such as an ion-exchanger, seems to be a generally applicable approach to deal with organic and inorganic interferences.

The described method is linear over almost four orders of magnitude and allows the detection of phenol in environmental samples at the parts-per-trillion level with a recovery of approximately 90%. The applicability to the selective sample handling of anionic drugs in biomedical samples with ordinary UV detection is presently being studied.

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Appendix

Automated procedure. Equipment: Kontron Model 200 programmer, Kontron MCS 670 Tracer switching unit; analytical column, 10 cm × 3.0 mm I.D. CP-Spher C18. Eluent, 0.4 M sodium acetate (pH 5.0) Methanol (1:1); flow-rate, 0.4 ml min^{-1}. Precolumns: 8 × 4.6 mm I.D. and 10 × 3.0 mm I.D. (Aminex A-28); 20 × 4.6 mm I.D. (PRP_1), flow-rate, Pump A, as indicated below.

Time (min)	Flow-rate (ml min^{-1})	Call file no.	Event
0.00	5.0		Flush capillaries with sample
1.95	2.0		
2.00	2.0	83	Sample over PRP_1 (5 ml)
4.50	2.0	93	Reset V_3
4.51	2.0	86	Switch to water
4.52	5.0		Flush capillaries with water
5.45	2.0		
5.50	2.0	83	Flush PRP_1 with 3 ml water[a]
7.00	2.0	93	Reset V_3
7.01	2.0	86	Switch to NaOH, pH 11.5
7.02	5.0		Flush capillaries with NaOH
7.95	1.5		
8.00	1.5	82	On-line purification of NaOH
8.50	1.5	84	Equilibrate A-28
8.51	1.5	85	Flush capillaries with eluent
8.95	1.2		
9.00	1.2	83	Transfer sample from PRP_1 to A-28 (3.6 ml)
12.00	1.2	93	Flush A-28 with 1.2 ml NaOH
13.00	1.2	94	Desorb A-28 with 3 ml eluent
13.10	1.2	92	All precolumns reset
13.20	1.2	86 dur 2	Switch to 50% methanol
13.30	5.0		Flush capillaries with 50% methanol
14.20	3.0		
14.30	3.0	83	Regenerate PRP_1 with 18 ml 50% methanol
17.50	3.0	86	Flush PRP_1 with 8 ml water
20.50	1.5	93	Reset V_3
20.60	1.5	84	Flush A-28 with 4.5 ml water
23.50	0.0		Stop pump A
23.60	0.0	88	Reset all
23.70	0.0	End	

[a]10 ml for river water samples.

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