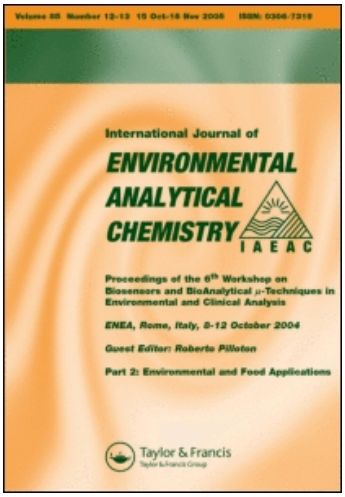

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On-Line Trace Enrichment, Cleanup and Determination of the Most Hydrophilic Priority Pollutant Phenols in Water

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ON-LINE TRACE ENRICHMENT, CLEANUP AND DETERMINATION OF THE MOST HYDROPHILIC PRIORITY POLLUTANT PHENOLS IN WATER

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An on-line trace enrichment method has been developed for the analysis of phenol, 2-nitrophenol, 4-nitrophenol, 2-chlorophenol and 2.4-dinitrophenol in water. The solutes are extracted and preconcentrated from water samples by solid-phase extraction on a reverse-phase copolymer precolumn. Thereafter, cleanup of the sample is performed by a two-step transfer of ionized phenols to an anion-exchange precolumn. The latter is further on-line analyzed by reversed phase chromatography with UV detection after each transfer step. Good recoveries, high precision and detection limits in the low µg/l concentration range have been achieved for the five phenols.

KEY WORDS: Priority phenols, on-line trace enrichment, water analysis

INTRODUCTION

The widespread use of phenolic compounds in the manufacture of a great variety of products (plastics, dyes, pesticides, disinfectants, etc.) and their presence in the direct discharge of industrial wastes into water streams have resulted in the pollution of natural and potable waters. With increased governmental pressure to reduce phenol concentration in effluent streams it has become essential to have more reliable and sensible methods of analysis for these compounds. Current standard methods of phenol determination in water (i.e. EPA-method 604) include liquid-liquid extraction of the sample, solvent evaporation and further cleanup or solute derivatization prior to GC-analysis[1]. The extensive sample handling in these long and laborious methods increases the risk of sample loss during the process resulting in low recoveries and poor precision.

In recent years, a number of methods based on solid-phase extraction techniques for the analysis of trace amounts of phenols in water have been described. Small precolumns packed with reverse-phase adsorbents have been used for the on-line trace enrichment of chlorophenols in natural waters[2–6]. Very polar and water-soluble polyhydroxybenzenes have been preconcentrated on a porous graphitized carbon adsorbent[7]. A two-step procedure for the determination of sub-ppb levels of phenol in water samples was presented by Nielen *et al*[8]; phenol was first preconcentrated on a relatively long precolumn packed with PRP1 copolymer, then, it was transferred to a small anion-exchange precolumn which was finally on-line analyzed by reversed phase chromatography with fluorescence detection. A very fast and interesting microextraction

technique was described by Buchholz *et al*[9] for the analysis of EPA phenols in aqueous samples; target analytes were extracted into the solid-phase coating of a silica fiber support, which was transferred to a gas chromatograph for the thermal desorption and analysis of the sample. A drawback of this technique is the lack of selectivity of the adsorbent which, in the case of real samples, adsorbs many other species that interfere with the extraction or the analysis of phenols.

In the methods reported so far, we have not found the application of on-line solid phase extraction to the determination of the group of most hydrophilic priority pollutant phenols, i.e.: phenol, 2-nitrophenol, 4-nitrophenol, 2-chlorophenol and 2.4-dinitrophenol, in water. The preconcentration of these five compounds on reverse-phase adsorbents is rather difficult due to their low hydrophobicity. Therefore, to extract these phenols from a water sample volume large enough to achieve good detection limits, a long precolumn must be used. However, a precolumn of large dimensions cannot be on-line analyzed if excessive band broadening of the solutes during their transfer to the analytical column is to be avoided[10]. An interesting approach that has been used in similar cases, where solutes with acid-base properties are involved, consists of a selective transfer of the ionized solutes from the long reverse-phase precolumn to a second smaller precolumn packed with an ion exchanger[8,11]. This procedure allows the simultaneous accomplishment of solute band compression and cleanup of the extract.

Using this approach, we propose in this paper an on-line methodology for the simultaneous determination of the five most hydrophilic priority pollutant phenols at low µg/l concentration in different water matrixes. Preconcentration is carried out in a long precolumn packed with a polymeric reversed phase. A cleanup procedure is then performed by a two-step transfer of ionized phenols to a small anion-exchange precolumn. Elution of the two sample fractions and LC separation and analysis are done isocratically on a C-18 analytical column with UV detection.

EXPERIMENTAL

Apparatus

Percolation of water samples and other solvents through the precolumn system was carried out with a Beckman 110B isocratic pump (sample pump), equipped with a manual six-channel selector valve placed at the pump inlet. Precolumn elution and HPLC analysis were performed with a LC system consisting of a Varian 5000 chromatograph (LC-pump), a Varian UV-100 spectrophotometer with the wavelength set at 270 nm and a Hewlett-Packard 3396A integrator. A 7125 Rheodyne valve with a calibrated 24 µl loop was used for the injection of phenol standard mixtures. Loop calibration was performed *in situ* as described in a precedent work[12]. Two 7000 Rheodyne valves, with the preconcentration and the cleanup precolumn respectively placed in the sample loop position, were inserted between the injector and the HPLC column for the column switching operations. The switching of all valves was manually controlled. The experimental setup is shown in Figure 1.

Stationary phases and columns

A 30 × 4.6 mm I.D. home-made stainless steel precolumn, slurry packed (pressure 207 bar) with a 30% w/v methanolic slurry of 10 µm styrene-divinylbenzene copolymer

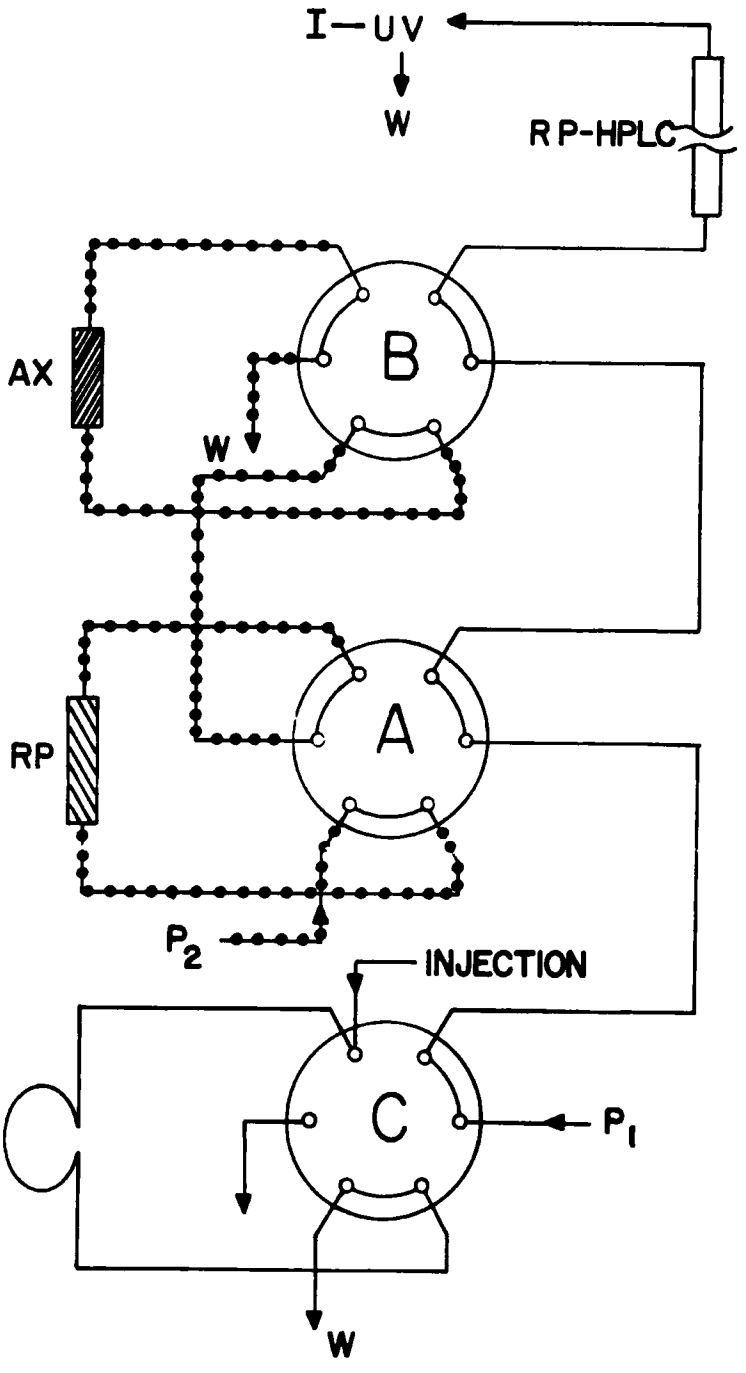

Figure 1 Experimental setup for the on-line preconcentration-analysis procedure. P_1, LC-pump; P_2, sample pump; A and B, high pressure switching valves; C, injector valve; UV, detector; I, integrator; W, waste. Precolumns: RP, 30 × 4.6 mm I.D. CHP-3C reverse-phase copolymer; AX, 20 × 2 mm I.D. PRP-X100 anion-exchange resin. Analytical column: RP-HPLC, 150 × 4.6 mm I.D. packed with 5 μm Spherisorb ODS-2. For illustration purposes the figure shows valves A and B in the "load" position with the two precolumns coupled in series.

CHP-3C from Mitsubishi was used for trace enrichment (RP precolumn in Figure 1). The cleanup precolumn (AX precolumn in Figure 1) was a commercial 20 × 2 mm I.D. stainless steel precolumn from Upchurch Scientific which also was slurry packed (pressure 117 bar) with a 30% w/v methanol-NaOH (pH 11.5) 80:20 v/v slurry, of 10 μm resin-based anion exchanger PRP-X100 from Hamilton. The analytical column (RP-HPLC column) was a 150 × 4.6 mm I.D. stainless steel cartridge prepacked with 5 μm Spherisorb ODS-2 from Phase Separations.

Mobile phase

The mobile phase used for the on-line elution of solutes from the anion-exchange precolumn and their HPLC separation was a mixture of acetonitrile, 20% v/v, and an aqueous buffer containing formic acid 0.05 M and perchloric acid 0.02 M adjusted to pH 3.5 with a NaOH solution.

Chemicals

HPLC grade acetonitrile (Prolabo) and type-1 reagent water from a Nanopure deionizer (Barnstead Thermolyne) were used to prepare mobile phases. Other chemicals: methanol (Prolabo), formic acid (Merck), perchloric acid (Aldrich) and sodium hydroxide (Merck) were analytical grade reagents.

All phenols were purchased from Chem Service with a degree of purity 98–99%. Acidity constants of the five phenols are as follows: phenol 9.99, 2-nitrophenol 7.23, 4-nitrophenol 7.16, 2-chlorophenol 8.52 and 2.4-dinitrophenol 3.94. Stock phenol solutions (1000 μg/l) were prepared by weighing and dissolving each solute in methanol. Standard mixtures of phenols at different concentrations in reagent water were prepared from stock solutions. These mixtures were used to spike water samples and for direct loop injection to calculate solute recoveries.

Procedure

In the group of compounds studied in this work, phenol itself is the most hydrophilic and has the highest pKa. Thus, this solute is the less retained in both adsorbents, the reverse-phase copolymer and the anion-exchange resin. On the other hand, 2.4-dinitrophenol in its neutral form has the strongest retention in reversed phases but its pKa is the lowest. Therefore, these two solutes were used to establish the conditions for sample preconcentration and cleanup.

First, the breakthrough of phenol from both precolumns was determined: For the anion-exchange precolumn, the experimental setup (Figure 1) was slightly modified by directly coupling valve B to the UV detector through the waste exit port. The RP-precolumn was kept off-line, maintaining valve A in the "inject" position and pump P_1 turned off. Valve B with the AX-precolumn was switched to the "load" position. Several sodium hydroxide solutions of different pH, containing phenol at 0.1 mg/l concentration, were percolated through the AX-precolumn and the elution profiles of the solute (breakthrough curves) were recorded. Breakthrough volumes were determined at 1% of the height of breakthrough curves.

For the reverse-phase precolumn, breakthrough volumes were estimated using the method proposed by Hennion *et al*[13] and the setup shown in Figure 1. The detailed description of the experimental operation is given in the first 7 steps of the general procedure presented below and in Table 1; only the sample volume indicated in step 3 was changed. Briefly, the method used was as follows: increasing volumes of a perchloric acid solution of pH 2, containing each the same amount of phenol (0.2 μg), were percolated through the long RP-precolumn; after each loading, the preconcentrated phenol was transferred to the AX-precolumn and the latter was on-line analyzed. The corresponding chromatograms were recorded and phenol peak areas were measured. As long as the processed sample volume remained smaller than the breakthrough volume in the RP-precolumn, the amount of concentrated phenol was constant and so was its peak area. When breakthrough occurred, the amount extracted decreased as also did the peak area.

Afterwards, using the results from the above experiments, 2.4-dinitrophenol was loaded on the reverse-phase precolumn, transferred to the anion-exchange precolumn and on-line analyzed. This experiment showed that the conditions established for the trace enrichment and cleanup of phenol were not adequate for 2,4-dinitrophenol which was only partially recovered. A new set of experiments was carried out with this solute to establish the conditions for its analysis and in particular for its complete transfer from the reverse-phase precolumn to the anion exchanger.

The general procedure outlined below was finally adopted for an adequate analysis of the five phenols. The position of switching valves A and B and the status of pumps P_1 (LC-pump) and P_2 (sample pump) in each step of the method are reported in Table 1 (Figure 1 for reference). Pump P_1 only delivers the mobile phase to the analytical column circuit. Pump P_2 is used to deliver up to six different solvents, including the sample, into the precolumn circuit. Solvent changes in this circuit were always preceded by abundant rinsing of pump lines and tubing with the new solvent at high flowrate. During this operation, valves A and B were kept in the "inject" position and pump P_1 was turned off.

Table 1 Position of switching valves and status of pumps during the different steps of the method.

# Step—Operation	*Valve A*	*Valve B*	P_1	P_2
1) RP-HPLC conditioning	L	L	ON	OFF
2) RP conditioning	L	I	OFF	ON
3) RP loading and flushing	L	I	OFF	ON
4) AX conditioning	I	L	OFF	ON
5) RP to AX, 1st transfer	L	L	OFF	ON
6) AX flushing	I	L	OFF	ON
7) Analysis of sample cut #1	L	I	ON	OFF
8) AX conditioning	I	L	OFF	ON
9) RP to AX, 2nd transfer	L	L	OFF	ON
10) AX flushing	I	L	OFF	ON
11) Analysis of sample cut #2; RP flushing & regeneration	L	I	ON	ON
12) Injection of standard; RP conditioning	L	I	ON	ON

P_1: LC-pump, P_2: sample pump, RP: reverse-phase precolumn (valve A), AX: anion-exchange precolumn (valve B), RP-HPLC: analytical column, L: load, I: Inject

General procedure

RP: reverse-phase precolumn, AX: anion-exchange precolumn, RP-HPLC: analytical column, S_1: $HClO_4$ (pH =2), S_2: NaOH (pH = 11.5), S_3: acetonitrile-NaOH (pH = 11.5) 10:90 v/v.

1) Condition RP-HPLC with mobile phase.
2) Condition RP with 30 ml of S_1
3) Load RP with 20 ml of sample adjusted to pH 2 and flush it with 0.5 ml of reagent water.
4) Condition AX with 30 ml of S_2
5) Transfer first sample fraction from RP to AX with 3 ml of S_2
6) Flush AX with 0.5 ml of reagent water.
7) Analyze the first sample cut by on-line elution of AX with mobile phase.
8) Condition AX with 30 ml of S_3
9) Transfer second sample fraction from RP to AX with 10 ml of S_3
10) Flush AX with 0.5 ml of reagent water.
11) Analyze the second sample cut by on-line elution of AX with mobile phase (P_1). Simultaneously flush RP with 10 ml of reagent water and regenerate it with 15 ml of methanol (P_2).
12) Inject a standard for quantification (P_1). Simultaneously condition RP with 30 ml of S_1 (P_2).
13) Go to step 3 for the analysis of the next sample.

For the development of the method and to optimize all the experimental conditions, the aforementioned steps were performed by manually controlling the switching valves and pumps. However, the whole procedure can efficiently be automated for routine analysis purposes.

RESULTS AND DISCUSSION

The use of two precolumns, a long one packed with a polymeric reverse-phase adsorbent and a smaller one packed with an ion exchanger has been reported in several works for the on-line analysis of polar ionizable compounds. During the first stage of the procedure, the solutes of interest in their molecular form and other apolar or moderately polar compounds are extracted and preconcentrated on the low selectivity reversed phase adsorbent, while very polar (in)organic ions not retained by this phase are eliminated. Afterwards, an aqueous solvent of adequate pH is used to produce an ionization of the analytes which are then desorbed from the polymeric packing, transferred to the second precolumn and adsorbed on the ion exchanger. Other less polar and/or not ionizable solutes are left in the first precolumn which acts as a filter. At this stage, an effective cleanup of the sample from inorganic and organic interferences has been performed[11].

However, the optimization of conditions for the on-line analysis of several compounds with different polarity and hydrophobicity is rather difficult. In particular, the composition of the solvent used for the transfer step, and not only its pH, is crucial. This solvent must be strong enough to completely desorb all the target solutes from the reversed phase adsorbent, but not too strong to compromise the cleanup of the sample by desorbing other compounds trapped in this precolumn; at the same time, the solvent must

be sufficiently weak to avoid breakthrough of the analytes from the ion-exchange precolumn. The elimination of the transfer step by directly loading the sample in the second precolumn in order to simplify the procedure is not advisable. Natural and potable waters generally contain high concentrations of inorganic ions that reduce the capacity of the ion exchanger to retain the compounds of interest. In these conditions breakthrough volumes are small and it is not possible to attain low detection limits.

Breakthrough of phenol on different precolumns

The retention of phenol on the reverse-phase copolymer is relatively weak. Results from our studies show that even with the "long" 30 × 4.6 mm I.D. precolumn, breakthrough of phenol is perceivable when more than 5 ml of solution are loaded. However the phenol elution front seems to be spread over a large volume. It was observed that the phenol loss increased from 7% to only 15% when the volume of solution percolated through the precolumn was increased from 10 ml to 20 ml. Therefore, we considered that a good compromise between accuracy and sensitivity of the method was to preconcentrate 20 ml of the water sample.

In the small anion-exchange precolumn, the breakthrough volumes of phenol decreased from 20 ml to 5 ml when the pH of the alkaline solution was increased from 10.5 to 11.5. Although phenol is not totally ionized in solutions of pH 10.5, its retention on the anion exchanger is stronger that at higher pH. This can be explained as follows: first, it is well known that the presence of an adsorbent provokes a displacement of acid-base equilibria in solution, in this particular case phenol becomes a stronger acid in the presence of the anion exchanger (which adsorbs phenolate ions) and is probably fully ionized at pH 10.5 in the AX-precolumn. Second, because it is not longer a question of ionization, the factor controlling retention in this pH range is the competition between phenolate and hydroxide ions; as the concentration of the latter increases, the breakthrough volume of phenol dramatically decreases. However, the experiments carried out using NaOH solutions of pH 10.5 to transfer phenol from the RP-precolumn to the AX-precolumn resulted in low recoveries, indicating that this pH was not appropriate to completely desorb the solute from the reverse-phase adsorbent. In this case we have the reverse phenomenon, phenol becomes a weaker acid and requires a higher pH to be ionized in the presence of a packing that adsorbs the molecular form of the solute.

In conclusion, for the transfer of phenol the best results were obtained with a small volume of a NaOH solution of pH 11.5. A volume of 3 ml was used instead of 5 ml considering that the presence of other compounds in the sample generally provokes a slight decrease of breakthrough volumes.

Unfortunately, the aqueous NaOH solution was not strong enough to completely desorb the other phenols from the reverse-phase packing, even though at this pH all of them are fully ionized. Addition of a small volume of acetonitrile to the alkaline solution was not successful at any pH; breakthrough of phenol from the anion exchanger always occurred before the complete transfer of the other solutes. Therefore, it was necessary to fractionate the sample for the analysis of the whole group. Results from our experiments show that a first fraction, containing all the preconcentrated phenol, about 80% of 2-nitrophenol, 4-nitrophenol and 2-chlorophenol and approximately 10% of 2.4-dinitrophenol, is transferred from one precolumn to the other with 3 ml of a plain aqueous NaOH solution of pH 11.5. Then, in a second step, the last sample fraction remaining in the reverse-phase precolumn can be desorbed with 10 ml of a mixture acetonitrile-NaOH (pH 11.5) 10:90 v/v. In this fraction the principal component is 2.4-dinitrophenol, the most hydrophobic compound of the group.

The solvents used for the first and second transfers are rather weak. Thus, other ionizable and more hydrophobic compounds eventually present in real samples, like the six other phenols of the EPA group, remain trapped in the first precolumn an do not interfere in the determination of the target phenols. This means that the RP-precolumn must be cleaned and regenerated with a strong solvent at the end of each analysis (step 11 of the general procedure).

During these experiments it was observed that the flush of the anion-exchange precolumn with reagent water, before the on-line analysis of the first sample fraction, was a critical step. This flush is necessary to avoid deterioration of the C-18 analytical column by the high pH solution remaining in the AX-precolumn after each transfer. Flushing with 1 ml of water was enough to provoke a considerable loss of phenol, which is explained by the pH change induced by water, with the consequent protonation of phenol and its desorption from the anion exchanger. Restraining the flushing volume to 0.5 ml of water, the recovery of this solute was acceptable and no deterioration of the HPLC column was observed.

Sodium hydroxide solutions were always freshly prepared from analytical grade NaOH pellets. We found that when highly alkaline solutions stand in plastic containers for long periods, they probably extract plasticizers, additives or other compounds from the plastic surface. These extracted compounds are concentrated in the anion-exchange precolumn during its conditioning (steps 4 and 8) and give rise to extremely dirty chromatograms when the precolumn is eluted with the mobile phase. By preparing the NaOH solutions just before use, the contamination problems was resolved.

Elution of phenols from the anion exchanger and HPLC analysis

Initially, the elution of solutes from the anion-exchange precolumn and their separation in the C-18 analytical column were assayed with mixtures of acetonitrile and an aqueous formic acid buffer of pH 3.5. This pH was appropriate for a good separation of the five phenols; 2.4-dinitrophenol, the solute with the lowest pKa, elutes between 4-nitrophenol and 2-chlorophenol in these conditions. However, very broad any asymmetric peaks were obtained with this mobile phase, probably due to a low kinetics of desorption from the anion exchanger. Addition of perchloric acid to the aqueous solution and readjustment of pH to 3.5 resulted in a remarkable improvement of peak shape. Peak retention times were also affected (decreased) by the presence of perchlorate. We believe that perchlorate ions, which are known to have a very strong affinity for anion exchangers, act as excellent displacers of phenolate ions from the resin surface improving their desorption kinetics.

From the above discussion two direct consequences may be deduced: first, the calibrating standards used to calculate recoveries cannot be injected in the single HPLC column but in the two columns: anion exchanger plus analytical column, because both entities participate in solute retention, separation and peak shape. Second, to restore the adsorption capacity of the anion exchanger, the precolumn must be thoroughly regenerated after the elution of solutes with the mobile phase containing perchlorate ions. We found that 30 ml of the sodium hydroxide solution were sufficient to accomplish this regeneration (steps 4 and 8 of the general procedure) and to obtain reproducible results.

For the analysis of the five phenols of interest, we decided to use UV detection at 270 nm instead of electrochemical detection because the oxidation of nitrophenols is rather difficult and requires very high potentials (> 1.2 volts) for sensible detection. With those potentials, background noise and electrode pollution cannot be adequately controlled.

Table 2 shows the accuracy and precision obtained from the analysis of nine identical water samples using the setup of Figure 1 and the procedure described in the experimental section. Samples were prepared from reagent water spiked with the standard mixture of phenols to give concentrations of 10 μg/l for each phenol. Figure 2 shows the chromatograms recorded from the on-line elution of the anion-exchange precolumn after the first and the second transfer steps. In chromatogram "A" the five phenols are present while in chromatogram "B" there is a large peak corresponding to 2.4-dinitrophenol and three small peaks due to residues of 4-nitrophenol, 2-chlorophenol and 2-nitrophenol. For quantitative calculations, the area of peak 1 in chromatogram "A" was used to determine phenol; the other compounds were determined by addition of the areas of their corresponding peaks in the two chromatograms. Recoveries were calculated by comparison with the peak areas obtained from a direct loop injection of the standard. Table 2 shows that the four less hydrophilic phenols are practically completely recovered. For phenol the recovery is only 85% because the volume of sample loaded in the RP-precolumn exceeds the breakthrough volume of this solute. Nevertheless, the precision of recovery for the five compounds of interest is excellent at this concentration level.

The linearity of the method was verified from the analysis of reagent water samples spiked with phenols at 9 different concentrations, in the range from 2.5 to 125 μg/l. For the five compounds the *peak area* vs *concentration* curve is linear in this concentration range with correlation coefficients 0.999. Recovery calculations for these experiments are reported in Table 3 as relations of *recovered amount* vs *added amount*. The intercepts of the linear regression equations are statistically equal to zero for the five phenols. On the other hand, with the exception of phenol, the slopes are equal to unity. This confirms the accuracy of the method in the range of concentrations studied. In this range, the recoveries of 2.4-dinitrophenol, 4-nitrophenol, 2-chlorophenol and 2-nitrophenol are about 100%, while for phenol the recovery oscillates between 84% and 88%.

Table 3 also reports the detection limits of this method. These values correspond to the compound concentration in reagent water that produces a signal of 3-times the baseline noise.

Figures 3 and 4 show chromatograms obtained from the on-line analysis of 20 ml of source water and highly polluted river water from an industrial site, respectively. The chromatograms from the same samples spiked at 10 μg/l of each phenol are also shown in the figures and the corresponding recoveries are reported in Table 4.

The analysis of the first source water fraction in Figure 3A shows the presence of some compounds that were not eliminated during the pretreatment process, indicating

Table 2 Accuracy and precision of the method. Conditions: 20 ml reagent water samples spiked at 10 μ/l of each phenol, analyzed using the setup of Figure 1 and the general procedure described in experimental. Results are the average from 9 independent samples. Data based on peak area measurements.

Compound	*1° transfer* *% Recovery*	*2° transfer* *% Recovery*	*Total* *%R**	*%RSD*
Phenol	85.5	–	85.5	1.2
4-nitrophenol	80.2	20.2	100.4	1.4
2.4-dinitrophenol	8.1	89.9	98.0	2.2
2-chlorophenol	80.4	18.5	98.9	1.9
2-nitrophenol	75.3	24.6	99.9	1.5

* %R = % total recovery

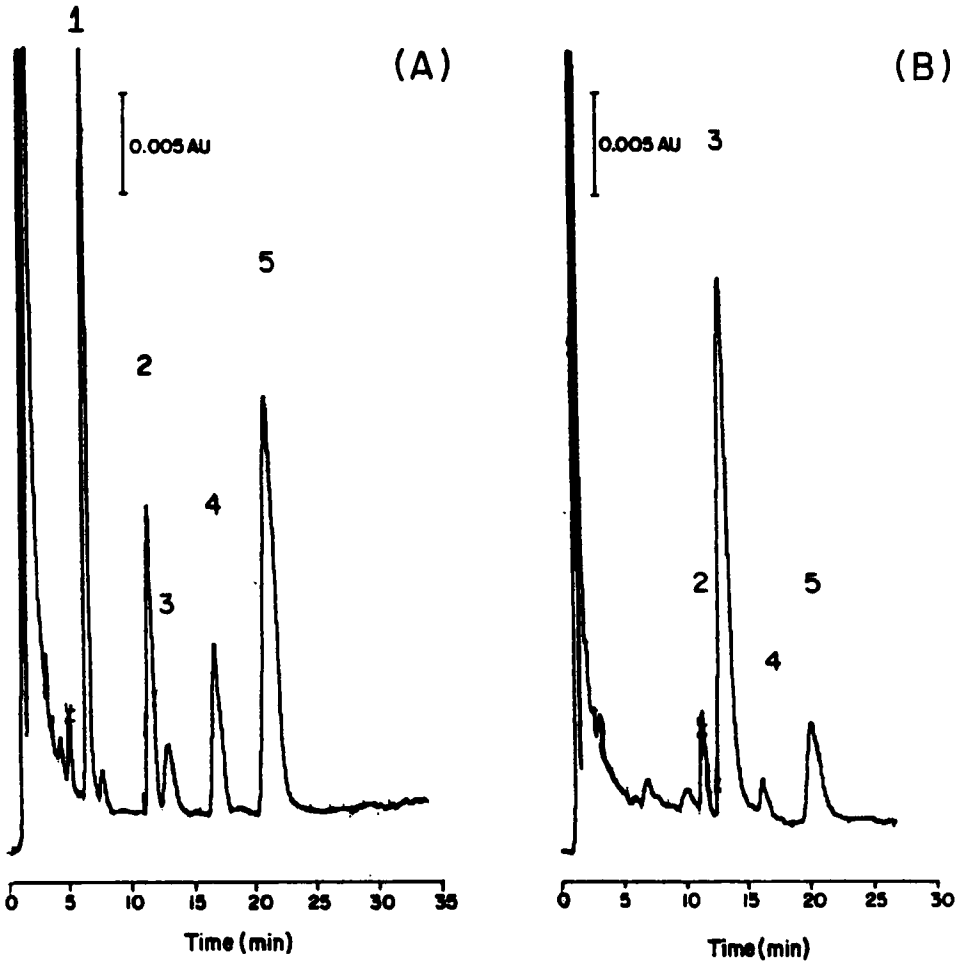

Figure 2 Chromatograms corresponding to the preconcentration and on-line analysis of 20 ml or reagent water fortified at 10 µg/l of each phenol and adjusted to pH 2. Elution of the first (A) and the second (B) fraction of the sample. Solutes: (1) phenol, (2) 4-nitrophenol, (3) 2.4-dinitrophenol, (4) 2-chlorophenol and (5) 2-nitrophenol. Eluent: acetonitrile-water (pH 3.5) 20:80 v/v, containing formic acid 0.05 M and perchloric acid 0.02 M, pH adjusted with NaOH. Flowrate 1 ml/min. UV detection at 270 nm. Other conditions as in Figure 1.

Table 3 Multilevel accuracy: recovered vs added amount and detection limits of the method. Conditions: 20 ml reagent water samples fortified with phenols at different concentrations (n = 9), analyzed using the setup of Figure 1 and the general procedure described in experimental.

Compound	*Intercept*	*SD** (*µg*)	*Slope*	*SD**	*Range* (*µg*)	*MDL*** (*µg/l*)
Phenol	–0.003	0.011	0.86	0.01	0.05–2.48	0.5
4-Nitrophenol	–0.006	0.023	0.99	0.02	0.05–2.32	1.0
2,4-Dinitrophenol	–0.002	0.018	1.01	0.01	0.05–2.38	1.0
2-Chlorophenol	–0.024	0.023	1.02	0.02	0.05–2.12	1.5
2-Nitrophenol	0.002	0.024	0.99	0.02	0.05–2.54	1.0

* SD = standard deviation

** MDL = method detection limit, defined for S/N = 3

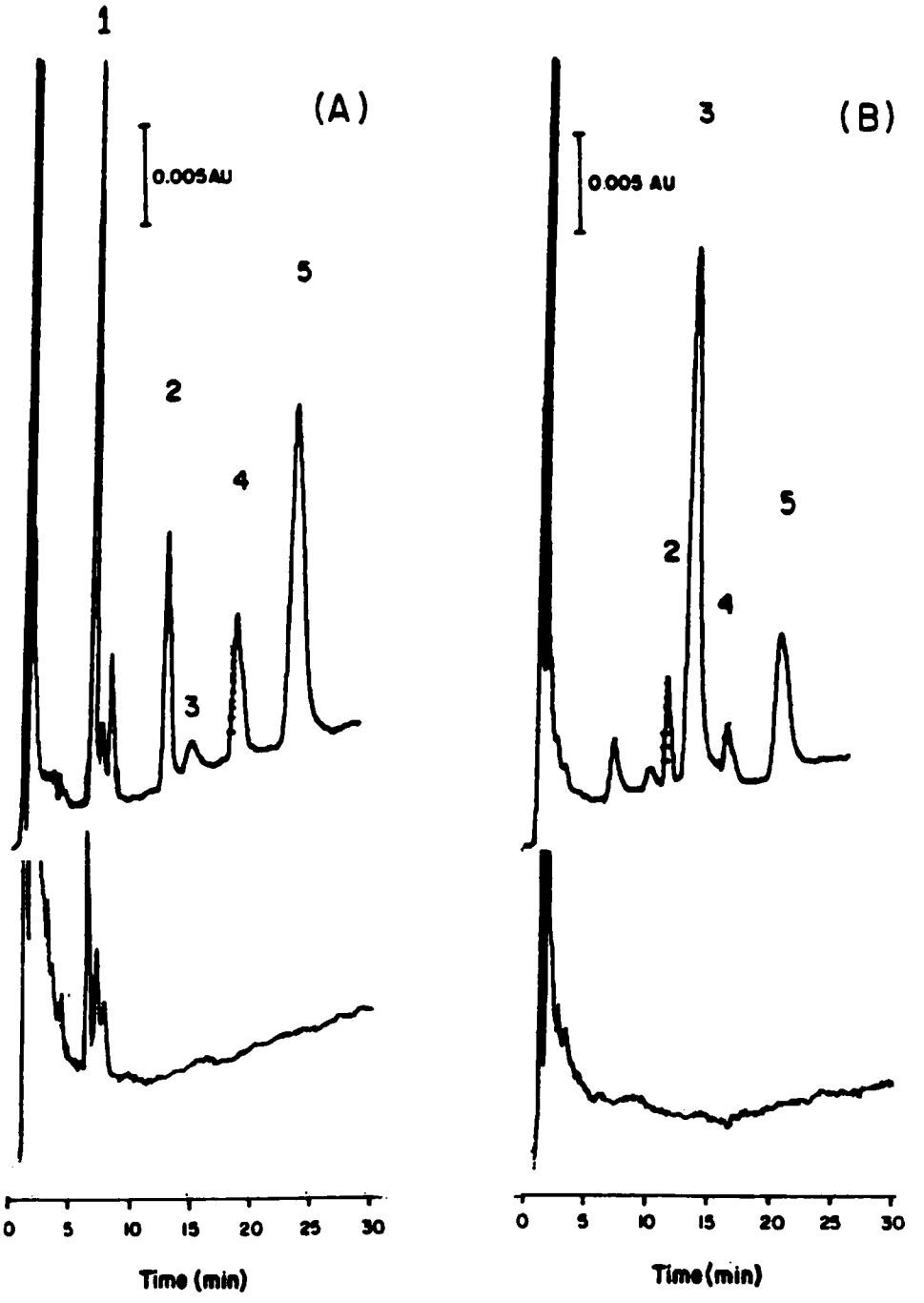

Figure 3 Trace enrichment and on-line analysis of 20 ml source water adjusted to pH 2 (lower chromatograms) and the same sample fortified at 10 µg/l of each phenol (upper chromatograms). Elution of the first (A) and the second (B) fraction of the samples. Same conditions as in Figure 2.

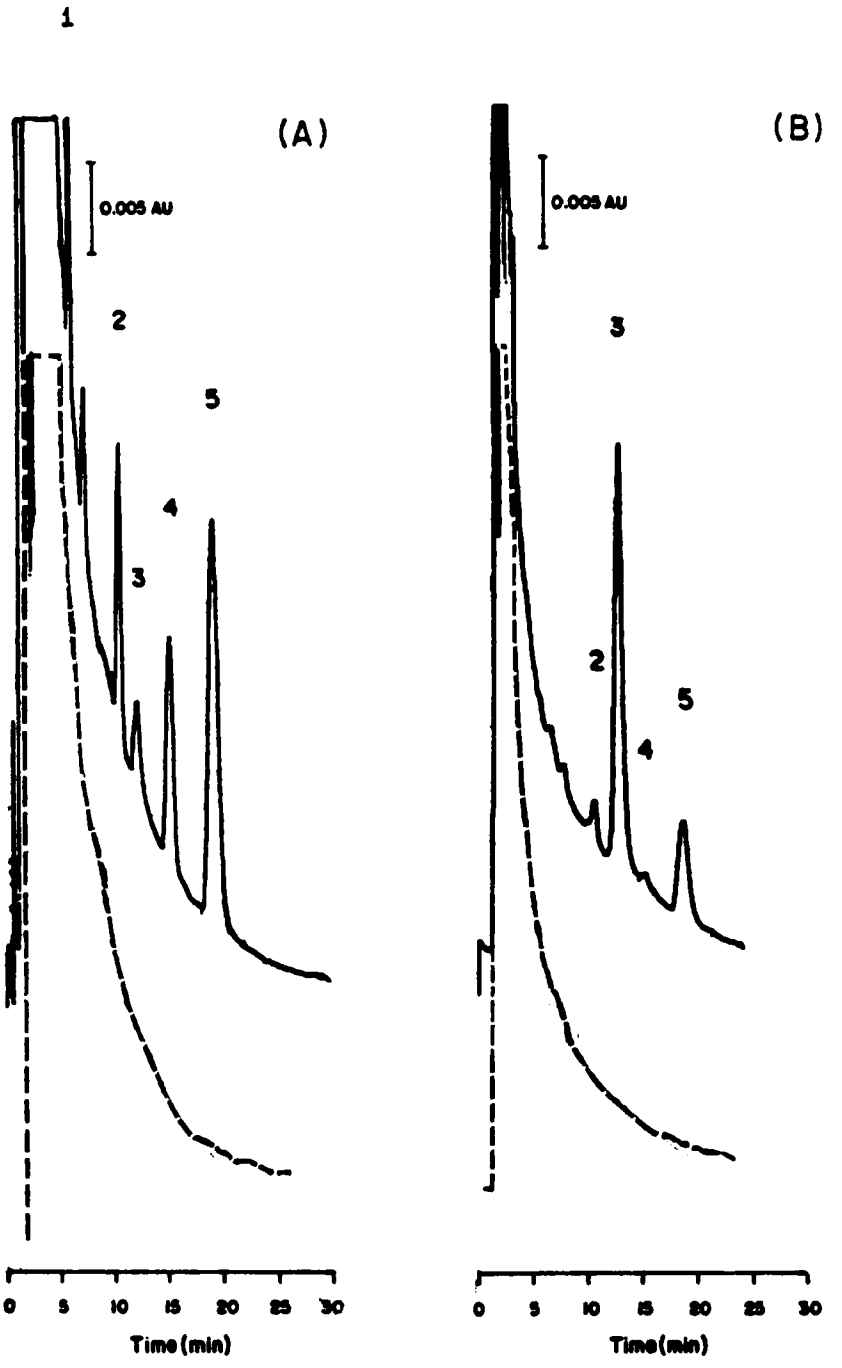

Figure 4 Chromatograms corresponding to the analysis of 20 ml polluted river water adjusted to pH 2 (dotted line) and the same sample fortifed at 10 μg/l of each phenol (solid line). Elution of the first (A) and the second (B) fraction of the samples. Same conditions as in Figure 2.

Table 4 Recovery rates in natural waters. Conditions: 20 ml water samples spiked at 10 µg/l of each phenol, analyzed using the setup of Figure 1 and the general procedure described in experimental.

Compound	*Source water %R**	*River water %R**
Phenol	121	96
4-Nitrophenol	100	87
2,4-Dinitrophenol	99	98
2-Chlorophenol	100	101
2-Nitrophenol	93	92

* %R = % total recovery

that they probably have acid-base properties and polarities similar to the hydrophilic phenols. By comparison with the analysis of the spiked sample, it was deduced that one of the compounds corresponded to phenol, although absolute confirmation can only be afforded by spectroscopic analysis, which were not carried out. Quantitative calculations using the peak area and considering a mean recovery of 86% permitted us to estimate a phenol concentration of 4 µg/l in the original sample. On the other hand, the recoveries of the analytes in the fortified source water are similar to those reported in Table 2 for spiked reagent water, except for phenol which is higher because it was already present in the sample. Hence, the accuracy, precision and detection limits of the method determined using reagent water remain valid for natural waters with simple matrixes as this one.

The chromatograms obtained from the analysis of river water (Figure 4) only show a big matrix peak at the beginning. This indicates not only the absence of the five hydrophilic phenols in the sample but also the excellent selectivity of the method, demonstrated by the efficient removal of interferences in this highly polluted water. In the fortified sample, the recoveries of the last eluting solutes are similar to those obtained with reagent and source waters, but the recoveries of phenol and 4-nitrophenol are significatively different. This is probably due to the wide matrix peak in chromatogram A that hinders the correct integration of the first peaks. The problem can be avoided by using a weaker mobile phase in order to increase the retention times of these compounds.

CONCLUSIONS

Trace enrichment and an efficient sample cleanup can be achieved using on-line precolumn technologies for the analysis of the most hydrophilic priority pollutant phenols in water. The combination of a non selective an adsorbent like a styrene-divinylbenzene copolymer with a selective material such as an ion exchanger seems to be a generally applicable sample pretreatment procedure for the determination of ionizable polar or moderately polar compounds in water. The sensitivity and selectivity provided by these methods give the possibility to attain good detection limits with simple UV detectors. The method proposed in this work, also provides other interesting advantages:

- A small sample volume, less than 50 ml, is required for the analysis, facilitating sample collection and transport.

- Sample manipulation is minimal, hence the risk of losses and/or contamination is reduced and the analysis of the five phenols can be achieved with an excellent precision.
- All the on-line operations can be easily automatized and made suitable for routine screening of series of samples.

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