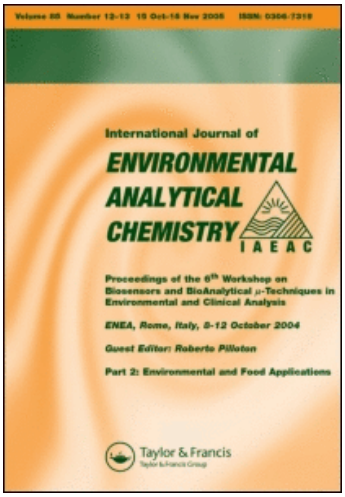

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TRACE LEVEL CHROMATOGRAPHIC DETERMINATION OF PRIORITY POLLUTANT CHLORO AND NITROPHENOLS IN WATER WITH ON-LINE SAMPLE PREPARATION

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A methodology for the on-line sample preparation and HPLC determination of priority pollutant chloro and nitrophenols at very low concentrations in water was developed. A long precolumn packed with a polymeric reversed phase is used for the extraction and preconcentration of the neutral analytes from an acidified water sample. Then, a selective transfer of the ionized phenols to a small anion-exchange precolumn is carried out by means of an alkaline hydroorganic solution. Finally, the coupling of the second precolumn to the HPLC system, equipped with UV and coulometric detectors, allows the on-line gradient elution, RP-separation and sensitive detection of the compounds of interest. Good recoveries, high precision and detection limits at the sub-μg/L level have been achieved with spiked water samples.

Keywords: Water analysis; on-line sample pretreatment; chlorophenols and nitrophenols

INTRODUCTION

Phenolic compounds are widely used as raw materials or as intermediates in the manufacturing of a great variety of products such as pharmaceuticals, plastics, dyes, pesticides, etc. They are also generated as subproducts in several industrial process like petroleum refining, pulp and paper production, coal gas liquefaction and many others. The presence of phenols, even at trace levels, in the waste effluents of the industry is of great concern because these compounds, and specially the chloro and nitrophenols, are toxic for the living organisms including

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the human being. Thus, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol are suspected of being carcinogenic. It is known that the toxicity of phenols increases with their acidity, therefore nitrophenols and polychlorophenols are the most toxic. However, the former are more easily biodegraded in the environment by reduction to their corresponding amines, on the contrary, chlorophenols are highly persistent. Considering this, the USEPA and other environmental agencies have included eleven phenols in the list of priority pollutants, among them there are five chlorophenols and four nitrophenols.

To prevent the pollution of natural waters with phenols or other toxic products, it is essential to have not only more sensitive methods of analysis but more reliable, simple and if possible automatizable ones, allowing a frequent monitoring of water streams. At present, the traditional long and laborious methods of sample preparation based on liquid-liquid extraction are being successfully replaced by the more rapid and economical solid-phase extraction (SPE) techniques. Indeed, the on-line SPE mode, which can be easily automated, has become one of the most powerful alternatives for the analysis of organic micropollutants in aqueous matrixes. [1,2,3]

From the early 80's, the application of SPE, on-line coupled to HPLC with UV, electrochemical or fluorescence detection, for the analysis of phenol[4], chlorophenols[5,6] and polyhydroxybenzenes[7] has been reported. In a recent work[8], we also proposed an on-line methodology for the simultaneous determination of the five most hydrophilic priority pollutant phenols in water. Phenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and 2-chlorophenol were extracted, preconcentrated and selectively isolated from water samples using an on-line system with two precolumns, one packed with a polymeric reversed phase and the other with an anion exchanger. This combination of adsorbents, one of great capacity but poor selectivity for the extraction and preconcentration of the analytes and the other more selective for the cleanup of the extract, was previously proposed in the work of Nielen et al[4]. Afterward, this type of system proved to be an excellent and generally applicable sample pretreatment procedure for the selective and sensitive determination of polar or moderately polar ionizable compounds in water.[9,10,11]

Initially, the purpose of this work was to continue the previous one with the development of a method for the simultaneous determination of the nine priority pollutant chloro and nitrophenols, using the two-precolumn system. However, because of the extreme hydrophobicity of pentachlorophenol compared with the other chloro and nitrophenols, it was not possible to achieve good results for all the compounds with the same experimental conditions. Therefore, the method was optimized for the efficient recovery, good accuracy and precision in the trace level determination of eight phenols.

EXPERIMENTAL

Apparatus

Figure 1 shows the diagram of the experimental setup. It was composed of two systems, one for sample pretreatment and the other for HPLC analysis, coupled through a 7000 Rheodyne valve (valve C). The sample pretreatment system consisted of a Beckman 110B isocratic pump (sample pump, P_2) with a manual six-channel selector valve (S) adapted to the pump inlet and two 7000 Rheodyne valves (valves A and B), with the preconcentration (RP) and the cleanup (AX) precolumns respectively placed in the position corresponding to the loop. The HPLC system included two Gilson, model 305 and 306 pumps (LC-pumps, P_1), a Gilson 805 manometric module, a Gilson 811 dynamic mixer, a 7125 Rheodyne injector (valve i) with an *in situ* calibrated[3] 24 µl loop, a reversed phase analytical column (RP-HPLC) and two different detectors in series: a coulometric detector (EQ) model Coulochem II from ESA, equipped with a 5011 analytical cell (EAC) and a 5020 guard cell (EGC), and a Spectromonitor 5000 diode array UV detector from Thermolyne Separations. Oxidation potentials of 850 mV and 900 mV (relative to the internal reference electrode of the cells) were set for the coulometric analytical and guard cells, respectively; the latter was placed between the dynamic mixer and the injection valve in order to lower the baseline noise and increase the sensitivity of detection by eliminating most of the oxidizable impurities present in the mobile phase. For the UV detector the wavelength was set at 280 nm. Chromatograms were recorded and integrated by Hewlett-Packard 3396A Series II integrators (I/G). Quantitation was always based on peak area measurements.

For the on-line coupling of both systems, valve C was inserted between the injector and the analytical column of the HPLC system and was connected to valves A and B of the sample pretreatment system. The switching of all valves, including the injector, was manually controlled.

Stationary phases and columns

The preconcentration precolumn (RP in Figure 1) home-made from Lichroma stainless steel tubing, 30 × 4.6 mm I.D., was slurry packed[8] with the 10 µm reversed phase copolymer MCI Gel CHP-3C from Mitsubishi. The cleanup precolumn (AX), 20 × 2 mm I.D. stainless steel from Upchurch Scientific, was slurry packed with the 10 µm anion exchanger PRP-X100 from Hamilton. The analytical column (RP-HPLC), 150 × 4.6 mm I.D., was also home-packed with 5 µm Spherisorb ODS-2 from Phase Separations. Separations were carried out at ambient temperature using a mobile phase gradient and a flowrate of 1 ml/min.

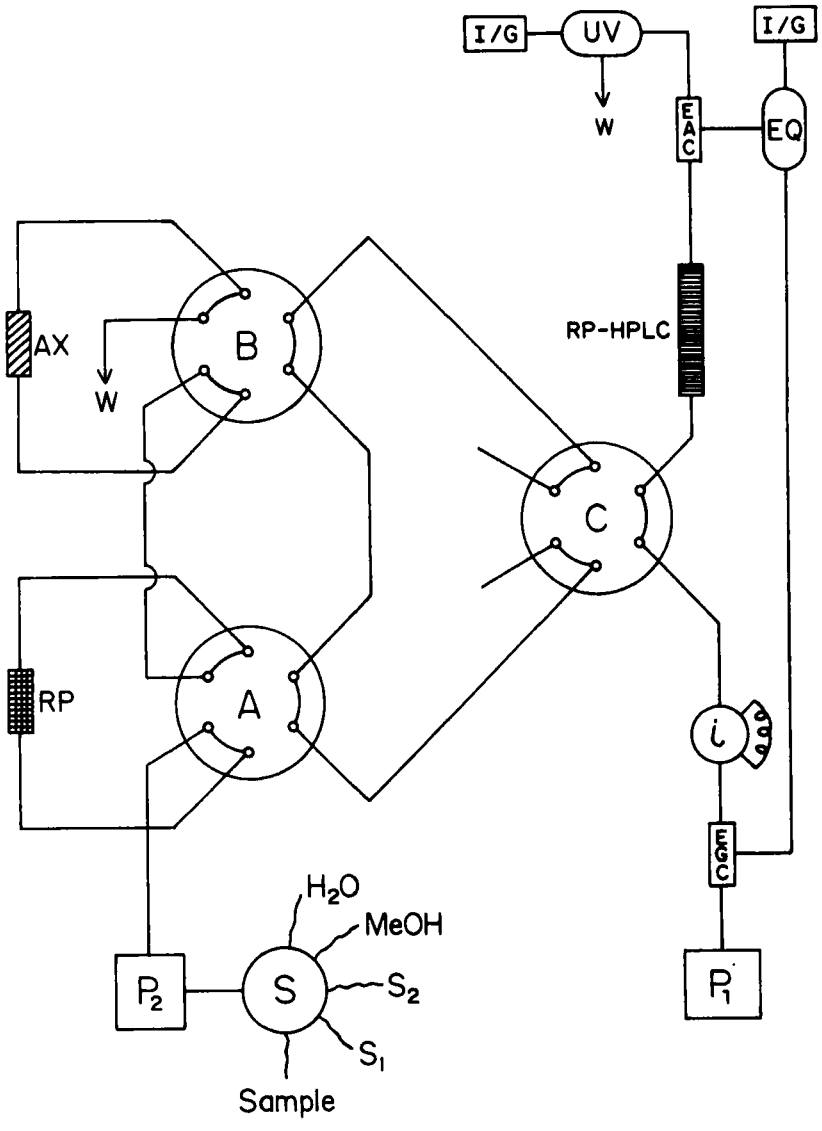

FIGURE 1 Experimental setup for the on-line sample preparation and HPLC determination of chloro and nitrophenols. P_1, LC-pumps; P_2, sample pump; A, B and C, high pressure switching valves; S, low pressure selector valve; i, loop injector; UV, diode array detector; EQ, coulometric detector equipped with a guard cell, EGC, and an analytical cell, EAC; I/G, integrators; RP, reverse-phase precolumn; AX, anion-exchange precolumn; RP-HPLC, analytical column; W, waste; S_1, $HClO_4$ (pH 2); S_2, acetonitrile-NaOH (pH 11.2) 10:90 v/v; H_2O, reagent water; MeOH, methanol. For illustration purposes the figure shows valves A, B and C in the "load" position

Eluents A and B were acetonitrile-water mixtures (16.5:83.5 and 70:30, v/v, respectively) containing perchloric acid 0.005 M and formic acid 0.045 M adjusted to pH 4.3 with NaOH. The gradient system was: Eluent B, 0 min = 0%, 15 min = 18%, 32 min = 55% and 40 min = 70%.

Chemicals

HPLC-grade acetonitrile and methanol were from Prolabo, Type-1 reagent water was obtained from a Nanopure deionizer (Barnstead Thermolyne), perchloric acid, formic acid and sodium hydroxide were analytical grade reagents obtained from various furnishers. All phenols were purchased from Chem Service with certified degree of purity between 96% and 99%. Acidity constants of the eight compounds studied are as follows, 2-nitrophenol (2NP): 7.23, 4-nitrophenol (4NP): 7.16, 2,4-dinitrophenol (2,4DNP): 3.94, 4,6-dinitro-2-methylphenol (4,6DN2MP): 4.35, 2-chlorophenol (2CP): 8.52, 2,4-dichlorophenol (2,4DCP): 7.85, 4-chloro-3-methylphenol (4C3MP): 9.55 and 2,4,6-trichlorophenol (2,4,6TCP): 6.42 . Other phenols also studied during this work but not included in the final method were pentachlorophenol (PCP) pKa 5.26 and 2,4-dimethylphenol (2,4DMP) pKa 10.58. Stock solutions (500 mg/l) were prepared by weighing and dissolving each phenol in acetonitrile. Standard mixtures of phenols at different concentrations in acetonitrile-eluent A, 50:50 v/v, were prepared from the stock solutions. The standards were used to spike water samples and for direct loop injection to calculate solute recoveries.

Procedure

Preliminary essays were carried out in order to determine the volume of sample that could be loaded in the RP precolumn and the volume and composition of the solvent for transferring the phenols from the RP to the AX precolumn. The following solutes were used: 4NP and 2CP, the least hydrophobic, 2,4,6TCP and PCP, the most hydrophobic, and 2,4DMP the weakest acid.

Breakthrough from the anion exchanger

The setup of Figure 1 was used. With valve A in the inject position and valves B and C in the load position, different volumes (5, 10 or 20 ml) and compositions (0% organic modifier, 10% acetonitrile or 40% methanol, v/v) of an alkaline solution at pH 11.2, containing a fixed amount of each solute in the volume assayed, were directly loaded in the AX precolumn by means of pump P_2. Meanwhile, pump P_1 was used to equilibrate the C-18 analytical column with eluent

A. After loading each sample, pump P_2 was turned off, the position of all valves was inverted and the mobile phase gradient was. run for the elution and analysis of the phenols. Recoveries were calculated by comparison of peak areas with those obtained from an injected standard containing the same amount of phenols as the samples.

Breakthrough from the reversed phase copolymer

In a similar way, different volumes (50 ml and 100 ml) and compositions (1% or 5% methanol, v/v) of an acidified water sample at pH 2, containing a fixed amount of each solute in the volume assayed, were loaded in the RP precolumn, transferred to the AX precolumn using optimized conditions deduced from the precedent experiments, and on-line analyzed for recovery determination. The detailed description of the experimental operation is given in the on-line operations section of the general procedure presented below and in Table I.

Recovery from the sampling bottle

For these experiments only the most hydrophobic solutes, 2,4,6TCP and PCP, were used. 200 ml of reagent water in an amber glass bottle were spiked with a known amount of the phenols to simulate a real sample. The sample was filtered through a nylon 66 membrane (0.4 μm pore diameter) and the bottle was rinsed with one of the following solvents: water (20 ml), NaOH pH 11.2 (20 ml) or methanol (2 ml) followed by NaOH pH 11.2 (20 ml). In each case the rinsing liquids were passed through the same filter and were collected in the same flask as the sample. The mixture was acidified to pH 2 and analyzed following the on-line operations section of the general procedure, except that the transfer solution used in steps 4 and 5 was methanol-NaOH (pH 11.2), 40:60 v/v, and the transfer volume was 20 ml. Recoveries were calculated considering the dilution of the original sample in each case.

From the three sets of experiments mentioned above it was recognized that pentachlorophenol and 2,4-dimethylphenol could not be analyzed with the rest qf the group, as will be discussed later. Thus, the following general procedure was finally adopted for the analysis of eight priority pollutant chloro and nitrophenols.

General Procedure

Off-line Operations

A 200-ml amber glass bottle with Teflon-lined cap, used as sampling bottle, is filled to the top with the water sample. Sample volumes, ranging from 195 to

205 ml, were later determined for quantitation. Filter the sample through a nylon 66 membrane (0.4 μm pore diameter) and rinse the bottle sequentially with 10 ml of NaOH (pH ≈ 11), 2 ml of methanol and again 10 ml of NaOH. Pass each rinsing portion through the same filter and collect it in the same flask as the sample. Acidify the mixture with 2 ml of $HClO_4$, approximately 1 M, and gently stir and sonicate for 3 min. The flask is directly used as reservoir of the sample pump.

The sample prepared in this way has a pH near 2.0 and contains about 0.9 %, v/v, of methanol. Its total volume is 224 ml.

On-Line Operations

RP: reverse-phase precolumn, AX: anion-exchange precolumn, RP-HPLC: analytical column, P_1 : LC-pumps, P_2: sample pump, S_1 : $HClO_4$ (pH 2), S_2: acetonitrile-NaOH (pH 11.2) 10:90 v/v.

1. Condition RP with 20 ml of S_1 (P_2)
2. Load RP with 50 ml of the prepared sample (P_2)
3. Flush RP with 0.2 ml of water (P_2). Begin the conditioning of RP-HPLC with eluent A (P_1).
4. Condition AX with 10 ml of S_2 (P_2). Continue the conditioning of RP-HPLC until the end of step 6 (P_1).
5. Transfer the analytes from RP to AX with 10 ml of S_2(P_2).
6. Flush AX with 0.2 ml of water (P_2).
7. Run the mobile phase gradient and analyze the sample by on-line elution of AX (P_1). Regenerate RP with 10 ml of water and 10 ml of methanol, then, condition RP with 20 ml of S_1 (P_2).
8. Condition AX and RP-HPLC with 20 ml of eluent A, then, inject a standard for quantitation and run the gradient (P_1). Load RP with 50 ml of the next sample (P_2).
9. Go to step 3 to continue the analysis of the next sample.

The position of switching valves A, B and C at each step of the method are reported in Table I. During the first two steps only pump P_2 is activated. From step 3 to 8, pumps P_1 and P_2 work simultaneously performing different tasks. A return order at step 9 closes a loop for the continuous analysis of samples. To stop analysis sequence, the conditioning and loading of the RP precolumn in steps 7 and 8 are not carried out and step 9 is deleted. The LC-pumps only deliver the mobile phases into the analysis circuit. The sample pump delivers five different solvents, including the sample, into the sample pretreatment circuit. Solvent changes in this circuit are preceded by a 10-ml rinsing of the pump head and tubing with the new solvent at high flowrate; this operation is carried out with valves A and B in the inject position and valve C in the load position. However, solvent changes at the interior of a same step (*ca.* step 7) are designed to be performed without rinsing of lines.

TABLE I Position of switching valves during the different steps of the method. RP: reversed-phase precolumn (Valve A), AX: anion-exchange precolumn (Valve B),. RP-HPLC: reversed-phase analytical column. L = Load, I = Inject

Step	*Operation*	*Valve A*	*Valve B*	*Valve C*
(1)	RP conditioning	L	I	L
(2)	Sample loading	L	I	L
(3)	RP flushing and Beginning of RP-HPLC conditioning	L	I	L
(4)	AX conditioning and RP-HPLC conditioning	I	L	L
(5)	RP to AX transfer and RP-HPLC conditioning	L	L	L
(6)	AX flushing and RP-HPLC conditioning	I	L	L
(7)	Sample analysis and RP regeneration and conditioning	L	I	I
(8)	Injection of a standard and Loading of next sample	L	I	I
(9)	Go to step 3			

In order to optimize all the experimental conditions of the method, the switching valves and pumps were manually controlled in this work. However, the whole *on-line operations* section can be easily automated for routine analysis.

The method includes a regeneration of the RP precolumn at each sample cycle because all the non polar and moderately polar compounds present in the sample that cannot be ionized and/or transferred to the AX-precolumn remain adsorbed on the polymeric reversed phase. The AX-precolumn and the analytical column are regenerated by the gradient elution. Nevertheless, we found that it was necessary to activate the anion exchanger after a 10-sample cycle by sequentially passing through the precolumn 20 ml of Na_2SO_4 0.05 M, 20 ml of NaOH pH 12 and 20 ml of reagent water. For a longer lifetime, the C-18 column should also be regenerated after the 10-sample cycle by an abundant rinsing with water followed by 10 to 20 ml of HPLC-grade acetonitrile.

RESULTS AND DISCUSSION

Breakthrough from the precolumns

The solvent used for the quantitative transfer of the phenols from the RP to the AX precolumn must be sufficiently strong and must have a pH higher than the highest pKa of the analytes in order to ionize and desorb them from the polymeric reversed phase. However, these two parameters must be carefully chosen

to avoid the breakthrough of some phenols from the AX precolumn during the transfer. Therefore, a study of retention in this precolumn was carried out prior to the coupling of the two precolumns.

TABLE II Breakthrough volumes in the anion-exchange precolumn. 2CP: 2-Chlorophenol, 4NP: 4-Nitrophenol, 2,4DMP: 2,4-Dimethylphenol, 2,4,6TCP: 2,4,6-Trichlorophenol, PCP: Pentachlorophenol

Organic Modifier	*Breakthrough Volumes (ml)*				
	2CP	*4NP*	*2,4DMP*	*2,4,6TCP*	*PCP*
none	>20	>20	>20	>20	>20
acetonitrile (10%)	10< <20	10< <20	10< <20	>20	>20
methanol (40%)	<5	<5	<5	>20	>20

The results of the experiments for the five compounds tested at different conditions in the AX precolumn are shown in Table II. The first conclusion from these results is that a pH of 11.2 seems adequate for a good retention of all the phenols in the anion exchanger. Breakthrough volumes were higher than 20 ml using a totally aqueous NaOH solution at this pH. On the other hand, the addition of an organic solvent to the alkaline solution provoked a remarkable decrease of breakthrough volumes for the least hydrophobic solutes and the weakest acid. The observed effect for 2CP (pKa 8.52) and 4NP (pKa 7.16) indicates that electric interactions and a reversed phase mechanism are both responsible for retention in the anion exchanger. However, the strong decrease in the retention of 2,4DMP (pKa 10.58) cannot be explained by reversed phase effects alone because this compound is considerably more hydrophobic than 2CP or 4NP. We believe that the observed behavior is mainly due to a significant decrease in the ionization degree of 2,4DMP and the consequent weakening of the electric interactions between the solute and the ion exchanger. Although all the phenols become weaker acids in the presence of organic modifiers, this effect is only reflected in the retention of compounds with a pKa close to the pH of the solution, as is the case of 2,4DMP. The final conclusion issued from the results in Table II is that the transfer of the analytes from the RP to the AX precolumn must be carried out with no more than 10 ml of a NaOH solution of pH 11.2, containing at most 10% of acetonitrile to prevent the breakthrough of some solutes during this step.

The above conditions were used in the set of experiments carried out to determine breakthrough volumes in the RP precolumn. A complete recovery of the

least hydrophobic solutes, 2CP and 4NP was only obtained when 50 ml of the acidified sample containing 1% of methanol were loaded in the RP precolumn. This means that breakthrough volumes for these compounds are between 50 and 100 ml for samples containing 1% of methanol and lower than 50 ml for samples with 5% of methanol. For 2,4,6TCP a total recovery was observed in all cases, indicating that its breakthrough volume from the RP precolumn is higher than 100 ml with samples containing either 1% or 5% of methanol. On the contrary, the recoveries of 2,4DMP and PCP were very low in all the experiments.

These results were surprising because 2,4DMP and PCP, being more hydrophobic than 2CP or 4NP, cannot be lost by breakthrough from the RP precolumn. Besides, Table II shows that neither are they lost by breakthrough from the AX precolumn during the transfer. Therefore, it is evident that the problem arises from an incomplete transfer of the two solutes. Indeed, separate experiments carried out with pentachlorophenol showed that this compound is better recovered (*c.a.* 80%) if a higher volume of a stronger solvent, like 20 ml of Methanol-NaOH (pH 11.2), 40:60 v/v, is used for the transfer step. In the case of 2,4DMP we could never obtain good recoveries, although the strength, the pH or/and the volume of the transfer solvent were increased.

In a precedent work[8] we discussed the fact that acidic compounds become stronger acids in the presence of adsorbents of their ionic form (anion exchangers) and become weaker acids in the presence of adsorbents of their molecular form (reversed phases). This is very well illustrated by the results obtained in this work with 2,4DMP. In an alkaline solution of pH 11.2 this solute is only partially ionized, but it has a good retention in the AX precolumn because the presence of the adsorbent of phenolate ions promotes the simultaneous ionization-adsorption phenomena. However, as discussed before, the addition of an organic modifier to the solution provokes the opposite effect and the retention of the solute in the AX precolumn decreases. On the other hand, when 2,4DMP is adsorbed on the reversed phase it becomes a weaker acid and cannot be ionized and desorbed with an aqueous solution of pH 11.2 . The addition of a small volume of organic modifier to the solution does not solve this problem because two opposite effects take place: the hydrophobic interactions decrease promoting desorption but the acidity of the solute also decreases rendering its ionization still more difficult. The other alternative is to increase the pH of the solution but in this case the subsequent adsorption of all phenolate ions, including 2,4DMP, on the anion exchanger is compromised by the high concentration of competing hydroxide ions and breakthrough from the AX precolumn rapidly occurs. Therefore, there is no satisfactory compromise for this weak acid.

From the results of this study it was concluded that the application of a two precolumn system (reversed phase plus ion exchanger) in the preconcentration

and cleanup of water samples containing ionizable organic compounds presents some limitations. One is for samples containing very weak acids or very weak bases that, definitively, cannot be treated in these systems. The other is for samples containing analytes with very strong differences in hydrophobicity. In this case, the two precolumn system can still be used, however the analytes cannot be determined simultaneously but by groups and different experimental conditions have to be optimized for each group.

Sample preparation

It is known that very hydrophobic compounds are easily adsorbed on the walls of vessels and the surface of filters or other materials that are in contact with their aqueous solutions. Therefore some precautions must be taken during the preparation of the sample prior to the preconcentration and cleanup procedures. The experiments carried out in this work showed that an important fraction of 2,4,6TCP and PCP remained adsorbed on the walls of the sampling bottle or on the filtering membrane when these materials were only rinsed with water. For improving solute recovery it was necessary to reduce the hydrophobic effects, which was done by rinsing with a NaOH solution and a small volume of methanol. In this way 2,4,6TCP was completely recovered but a significant part of PCP was still lost. A more efficient recovery of the latter should be possible by increasing the volume of methanol used to rinse the bottle and filter. Nevertheless, we did not tested it because the addition of more methanol to the sample decreases the volume of sample that can be preconcentrated in the RP precolumn, compromising the sensitivity of the final method.

The results from these experiments reinforce the conclusion about the impossibility of finding common conditions for the simultaneous analysis of pentachlorophenol and the other priority pollutant chloro and nitrophenols.

Elution of phenols from the anion exchanger and HPLC analysis

The elution from the AX precolumn and the chromatographic separation of the eight analytes finally included in the method were achieved with the slightly concave gradient reported in the experimental section. The pH of the mobile phases was fixed to 4.2 with the formic acid-formiate buffer (pKa 3.8). In these conditions, the two dinitrophenols are partially ionized and elute in positions one and five; all the other phenols elute in the order of increasing hydrophobicity (Figure 2).

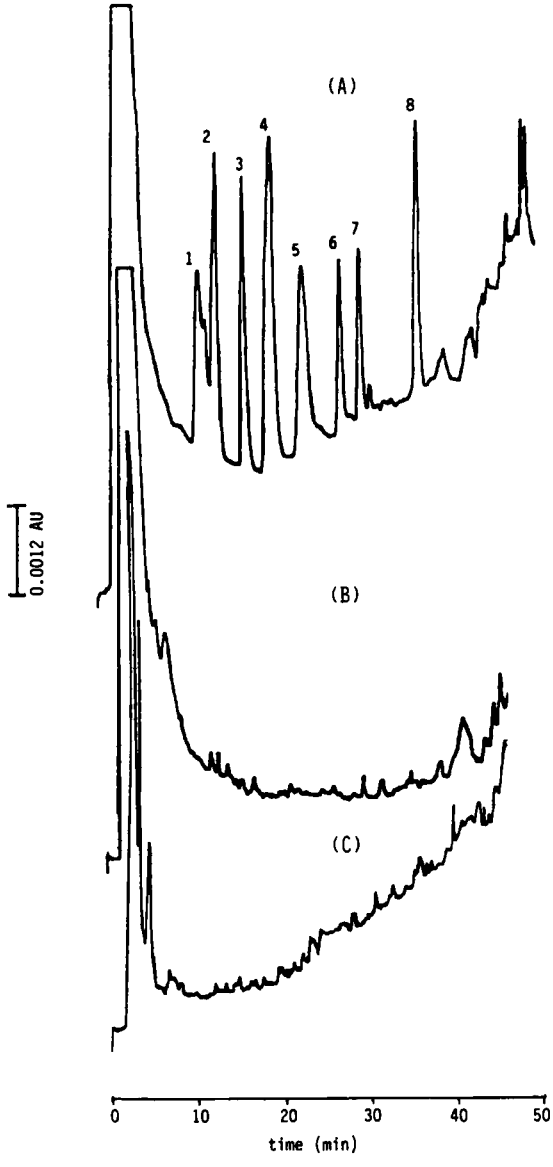

FIGURE 2 UV chromatograms corresponding to the analysis of water samples. (A) source water fortified at ~2.5 μg/l of each phenol, (B) blank of source water, (C) blank of reagent water. Solutes: (1) 2,4-dinitrophenol, (2) 4-nitrophenol, (3) 2-chlorophenol, (4) 2-nitrophenol, (5) 4,6-dinitro-2-methylphenol, (6) 4-chloro-3-methylphenol, (7) 2,4-dichlorophenol, (8) 2,4,6-trichlorophenol. Preconcentration of 50 ml of the prepared sample on the reverse-phase precolumn. Cleanup by transfer of solutes to the anion-exchange precolumn with 10 ml of acetonitrile-NaOH (pH 11.2) 10:90 v/v. Chromatographic conditions described in the experimental section

Perchlorate ions were also added to the mobile phases because their strong affinity for anion exchangers facilitates the desorption of the dinitrophenols from the AX-precolumn and improves their peak shapes. In the absence of perchlorate, these solutes have longer retention times and elute as very broad and asymmetric peaks. However, we remarked that after some sample cycles the retention capacity of the AX precolumn decreased, probably due to the blockage of ion-exchange sites by perchlorate anions which were not displaced during the conditioning of the precolumn with NaOH. Reactivation of the packing after a 10-sample cycle, as indicated in the experimental section, allows a complete regeneration of the ion exchanger.

The calibrating standards used to calculate recoveries were always injected with the AX precolumn coupled to the analytical column because both of them participate in solute retention and peak shape. The signal from the UV detector was used for the analysis of nitrophenols, which are difficult to oxidize. Chlorophenols were analyzed with the more sensitive electrochemical detector.

Table III shows the accuracy and precision of the method obtained from the analysis of eight identical reagent water samples spiked at ~2.5 μg/l of each phenol. The lowest recovery, 88.8%, corresponds to 2CP the least retained analyte in the AX precolumn. All other phenols are well recovered (> 90%) and the precision of recovery (<10%) is excellent for this concentration level.

TABLE III Accuracy and precision of the method. Conditions: reagent water samples and a source water sample were spiked at ~2.5 μg/l of each phenol and analyzed using the setup of Fig 1 and the general procedure described in experimental. Results in reagent water are the average from 8 independent samples. Data are based on peak area measurements

	Reagent Water		*Source Water*
Compound	*Recovery*	*RSD*	*Recovery*
	(%)		*(%)*
2,4-Dinitrophenol	95.3	5.7	101.9
4-Nitrophenol	98.7	4.3	94.6
2-Chlorophenol	88.8	4.9	80.5
2-Nitrophenol	100.7	5.0	97.2
4,6-Dinitro-2-methylphenol	95.3	6.4	91.5
4-Chloro-3-methylphenol	95.3	7.9	103.2
2,4-Dichlorophenol	95.7	7.3	95.5
2,4,6-Trichlorophenol	100.2	5.9	102.9

The multilevel accuracy of the method was determined from the analysis of reagent water samples fortified with the phenols at various concentrations, from ~0.25 to 20 or 40 μg/l. Phenol recoveries were calculated and analyzed as relations of *Recovered amount* Vs *Added amount.* The correlation coefficients of the linear regression equations for the eight phenols were ≥0.999. Table IV reports the corresponding intercepts and slopes. For a confidence level of 5%, all the intercepts are statistically equal to zero. The slopes, that represent the fraction of solute recovered, are: 0.87 for 2CP and ≥ 0.90 for the other phenols, with confidence intervals lower than ± 5%. These results demonstrate the linearity of the method in the range of concentrations studied and confirm its good accuracy and precision.

TABLE IV *Recovered Amount vs Added Amount.* Conditions: Reagent water samples fortified with phenols at different concentrations were analyzed using the setup of Figure 1 and the general procedure described in experimental. Data are based on peak area measurements

Compound	*n*	*Intercept* (μg)*	*Slope**	*Range (μg)*
2,4-Dinitrophenol	7	0.00±0.01	0.98±0.02	0.01–0.93
4-Nitrophenol	7	0.00±0.01	0.99±0.04	0.01–0.90
2-Chlorophenol	7	-0.00±0.01	0.87±0.02	0.01–0.90
2-Nitrophenol	7	0.00±0.01	0.99±0.02	0.01–0.98
4,6-Dinitro-2-methylphenol	8	-0.01±0.02	1.00±0.03	0.01–1.86
4-Chloro-3-methylphenol	8	0.01±0.01	0.90±0.01	0.01–1.80
2,4-Dichlorophenol	8	-0.01±0.02	0.99±0.03	0.01–1.78
2,4,6-Trichlorophenol	8	-0.01±0.02	1.01±0.02	0.01–1.76

* Intervals calculated for a confidence level of 5%. Correlation coefficients were ≥ 0.999 for the eight phenols.

The proposed method was applied to the analysis of a natural source water used as drinking water by countrymen. Figure 2 shows the UV chromatograms obtained from the analysis of the blank sample, the same sample fortified at ~2.5 μg/l of each phenol and, for comparison, the blank of a reagent water sample. The chromatogram of the blank source water (Figure 2B) only presents a big matrix peak at the beginning, some gradient peaks at the end and very small signals, at the level of the baseline noise, in the region where the phenols of interest elute. Indeed, the only significant difference between the chromatograms of the reagent water and the source water is the larger size of the matrix peak in the

latter which is due to organic matter, probably humic substances[12], present in this sample and not eliminated during the cleanup procedure. Therefore, the source water does not contain detectable quantities of chloro or nitrophenols. The chromatogram of the fortified sample (Figure 2A) shows that low µg/l concentrations of the phenols can be easily determined in this water. Phenol recoveries are comparable to those obtained with reagent water spiked at the same concentration as shown in Table III; only the recovery of 2CP is a little lower, probably because the presence of other organic compounds at higher concentration in the source water provokes a slight decrease of its breakthrough volume in the AX precolumn. Figure 3 illustrates the high sensitivity of this method for the analysis of chlorophenols. The coulometric detector chromatograms presented in the figure correspond to the analysis of the blank source water and the same sample fortified at ~250 ng/l of each phenol; the four chlorophenols are clearly detectable at this concentration level.

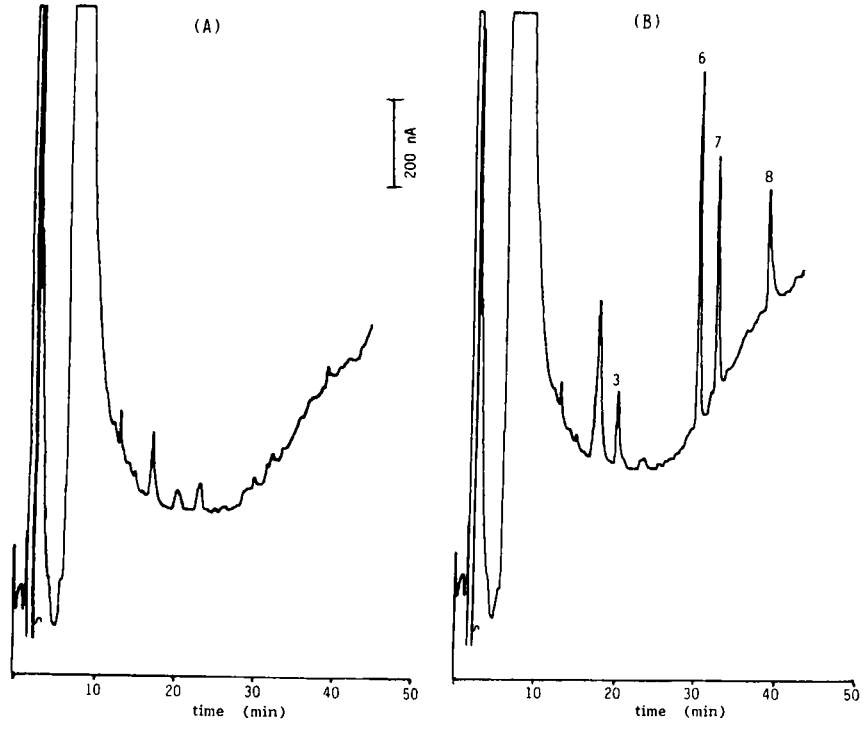

FIGURE 3 Coulometric detector chromatograms obtained from the analysis of the source water sample. (A) blank sample, (B) sample fortified at ~250 ng/l of each chlorophenol. Solutes: (3) 2-chlorophenol, (6) 4-chloro-3-methylphenol, (7) 2,4-dichlorophenol, (8) 2,4,6-trichlorophenol. Other conditions as in Figure 2

The detection limits of the method (MDL) were determined in reagent water and the source water by analyzing samples fortified with progressively decreasing concentrations of the phenols. Table V shows the MDL for chlorophenols and nitrophenols in both samples. These values correspond to the compound concentration that produces a signal of three times the baseline noise when the sample is preconcentrated and analyzed using the proposed method. The MDL for the group of chlorophenols is significatively lower than that for nitrophenols, which is due to the higher sensitivity of the coulometric detector compared to the UV detector.

TABLE V Detection limits of the method (MDL) in the preconcentration and on-line analysis of spiked reagent water and source water. UV detection at 280 nm for nitrophenols and electrochemical detection at 0.850 V for chlorophenols. Detection limits are defined for a signal-to-noise ratio of 3

Compound	*MDL in reagent water (ng/l)*	*MDL in source water (ng/l)*
2,4-Dinitrophenol	250	500
4-Nitrophenol	250	500
2-Chlorophenol	50	125
2-Nitrophenol	250	500
4,6-Dinitro-2-methylphenol	250	500
4-Chloro-3-methylphenol	25	25
2,4-Dichlorophenol	50	50
2,4,6-Trichlorophenol	50	125

Finally, we also tried to apply the method for the monitoring of trace concentrations of chloro and nitrophenols in the tap water of Mexico City. Figure 4 shows the coulometric detector chromatograms obtained from these experiments. Figure 4A is the chromatogram of the direct injection of the standard used to spike the sample, figure 4B corresponds to the analysis of the blank sample and figure 4C was obtained from the fortified sample which was analyzed just after being spiked. The obvious degradation of the phenols can be appreciated in the last chromatogram where several peaks, not present in the blank sample or in the standard, appear at the end of the gradient while the peaks of the phenols of interest are considerably reduced or totally vanished (*ca.* 2CP). This surprising result is attributed to the residual chlorine present in the sample. The official norm in Mexico for the concentration of this parameter in tap water is 0.5–1 mg/l; it has been published[13] that, at these chlorine concentrations, ppb levels of phenols

rapidly react by substitution of hydrogen atoms of the aromatic ring to form polychlorophenols, the continued reaction can even go until the breaking of the ring to form small aliphatic residues. Indeed, we have observed in some experiments the vanishing of all the peaks in the chromatogram with the exception of the matrix peak. In all the cases, the fortified samples were prepared in amber glass bottles and were analyzed, at most, two hours after their preparation. These interesting facts are currently being investigated in order to correlate the residual chlorine concentration in the tap water furnished at different periods of the year, and other parameters, with the observed chromatographic profiles.

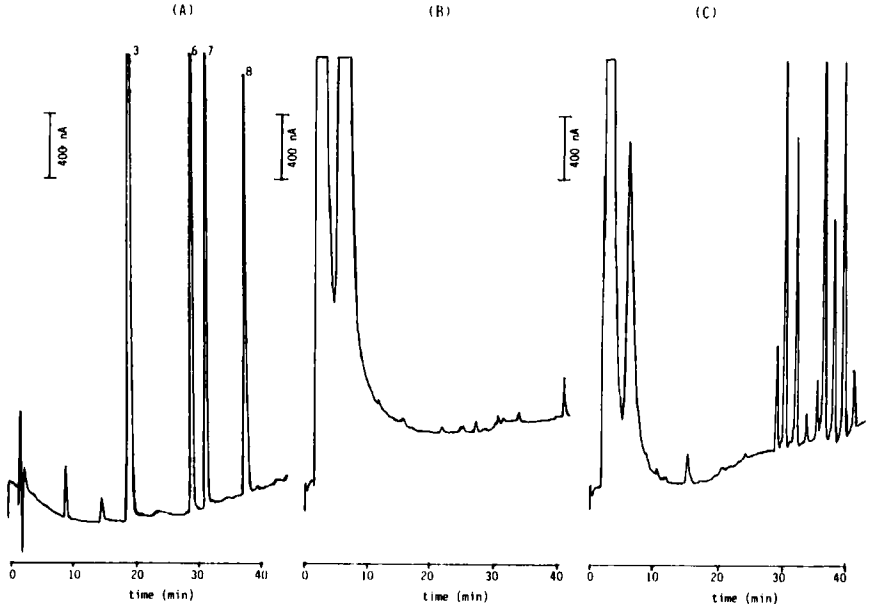

FIGURE 4 Analysis of tap water. (A) Direct injection of the standard (120 ng of each phenol), (B) blank of tap water, (C) tap water fortified with the standard at ~2.5 μg/l of each phenol. Solutes: (3) 2-chlorophenol, (6) 4-chloro-3-methylphenol, (7) 2,4-dichlorophenol, (8) 2,4,6-trichlorophenol. The fortified sample preconcentrated on the reverse-phase precolumn corresponded, theoretically, to 110 ng of each phenol. Other conditions as in Figure 2

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