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Trace determination of phenols in natural waters Extraction by a new graphitized carbon black cartridge followed by liquid chromatography and re-analysis after phenol derivatization

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Abstract

Based upon solid-phase extraction, a sensitive, selective and robust liquid chromatography (LC) procedure for determining the U.S. EPA eleven priority pollutant phenols in natural waters is presented. The method involves passing 4 and 1 l, respectively, of drinking and river waters through a reversible cartridge filled with 0.5 g of a new example of graphitized carbon black (Carbograph 4) at flow-rates of about 100 ml/min. After washings, phenols are eluted by reversing the cartridge and back-flushing it with 6 ml of a CH_2Cl_2 - CH_3OH mixture containing tetrabutylammonium chloride, 10 mmol/l. The eluate is then divided in two equal portions that are taken to dryness. After reconstituting the residue of one portion of the extract with a suitable solution, phenols are separated and quantified by ion-suppression, reversed-phase LC with UV detection. The goal of analyzing phenols with a low probability of false positives can be easily reached by converting phenols in the second portion of the extract to acetyl derivatives. The reaction mixture is then injected into the same analytical column and confirmation of the eventual presence of certain phenols in the sample is obtained by observing the disappearance of the peaks for phenols and the simultaneous appearance of peaks for the corresponding acetyl derivatives. By this method, the presence of 1.3 $\mu\text{g/l}$ of 2-chlorophenol in a surface water sample was ascertained. Recovery of phenols, including phenol itself, were higher than 90%. Conversion of phenols to acetyl derivatives was completed by reacting 40 μl of acetic anhydride for 6 min at 50°C, except for 4,6-dinitro-2-methylphenol (78% reaction yield).

Keywords: Water analysis; Graphitized carbon black; Environmental analysis; Solid-phase extraction; Phenols

1. Introduction

Phenols are toxic substances frequently occurring in the aquatic environment as a result of contamination from a variety of sources. Recent regulations enacted in many European countries state that phenols cannot be present in waters destined for human consumption at individual levels exceeding

100 ng/l. In order to assess whether a drinking water is in compliance with these regulations, analytical methods capable of detecting phenols at 20–30 ng/l are needed. Moreover, these methods should also be able to detect phenols at 1–3 $\mu\text{g/l}$ levels in surface water samples, as it has been ascertained that trace amounts of several phenols are toxic to aquatic life [1]. Many methods proposed in the past for determining phenols are not sufficiently sensitive and/or selective to comply with these requirements. For adoption by environmental laboratories in routine analysis, an analytical method for monitoring traces of phenols in natural waters needs to be sensitive and

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selective, and should also possess attributes of rapidity, simplicity and, last but not least, robustness.

For a number of well-known reasons, both off-line and on-line solid-phase extraction (SPE) by a variety of sorbent materials contained in small cartridges [2–10] or in membranes [11] has replaced liquid-liquid partitioning as the technique of choice for the enrichment of phenols.

Commercially referred to as Carbograp 1 or Carbopack, graphitized carbon black (GCB) has proved to be a valuable adsorbing material for the SPE of pesticides [12–14], chloroanilines [15], and linear alkylbenzene sulfonate surfactants and their biointermediates [16,17]. Years ago, the ability of a 250-mg Carbograp 1 cartridge to extract quantitatively the eleven U.S. EPA phenols from large water volumes was evaluated [18]. The recovery data obtained showed that this small cartridge was effective in removing the phenols considered, except for phenol, from 1 l of a ground water sample. 90% of phenol was found in the water effluent. Later on, by analyzing 150-ml aliquots of various river water samples, we also observed that the recovery of phenol was affected to an unexpected extent by the dissolved organic content (DOC) in the particular aqueous sample analyzed. Saturation effects of the Carbograp 1 trap by fulvic acids were responsible for this unwelcome effect. Another shortcoming of this method is that, in order to avoid irreversible adsorption of 2,4-dimethyl phenol on the GCB surface, two distinct solvent mixtures have to be passed through the GCB bed for re-extracting all of the eleven phenols. Recently, these problems were eliminated by adopting a 1-g Carbograp 1 reversible cartridge [19]. By this device, complete extraction of phenol from 2 l of drinking water was made possible. The problem of eluting some phenols that have a particularly large affinity for the GCB surface from the large-size GCB cartridge with a reasonable volume of the mobile phase was circumvented by reversing the cartridge prior to the desorption step. Although enabling determination of phenols to be performed at the ng/l level, this method has some drawbacks in that it lacks selectivity and, to an extent, robustness and rapidity. The selectivity of this method relies on diode array UV detection. However, except for nitrophenols, phenols do not give sufficiently distinctive UV spectra to allow their un-

ambiguous identification in complex matrices. Another shortcoming of the method cited above is that adoption of a large-size extraction cartridge leads to an increase in the sample extraction time. Finally, the method reported above lacks robustness, as it involves a critical step of extract concentration. To avoid evaporative losses of the most volatile phenols, which occur during partial solvent removal, they are converted in advance to non-volatile phenoxy ions by the addition of a suitable amount of KOH to the extract. Even under these conditions, however, the solvent removal step is still a critical one, as an excessive concentration of the extract can provoke loss of dinitrophenols by degradation in an excessively basic medium. Also, degradation of dinitrophenols was observed to occur more readily by concentrating extracts of river water samples. Fulvic acids, which are naturally occurring compounds, were responsible for this adverse effect [19].

As shown elsewhere [20], a new experimental example of GCB, commercially referred to as Carbograp 4, has proved to be more efficient than Carbograp 1 in extracting very polar compounds from large water volumes.

The objective of this work was to develop a robust and selective liquid chromatography (LC) method enabling rapid determination of the eleven U.S. EPA phenols in actual samples in the ng/l region. The method involved extraction of phenols by a reversible 0.5-g Carbograp 4 cartridge and reextraction by an eluent phase containing a quaternary ammonium salt. Before drying, the extract was divided into two portions. One was used for analyzing intact phenols by LC with UV detection. For confirmation of phenols tentatively identified, the second portion of the extract was injected into the same LC column after conversion of phenols to their corresponding acetyl derivatives.

2. Experimental

2.1. Reagents and chemicals

Authentic phenols were obtained from various sources. Individual standard solutions were prepared by dissolving 100 mg of each phenol in 100 ml of methanol. For recovery studies, a single working

composite standard solution was prepared by combining 1-ml aliquots of each of six individual stock solutions (4-nitrophenol, 2,4-dinitrophenol, 2-nitrophenol, 4,6-dinitro-2-methylphenol, 2,4-dichlorophenol and pentachlorophenol) with 2-ml aliquots each of five individual stock solutions (phenol, 2-chlorophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol) and diluting to a volume of 100 ml with acetonitrile.

For LC, distilled water was further purified by passing it through the Milli-Q Plus apparatus (Millipore, Bedford, MA, USA). Acetonitrile "Plus" of gradient grade was obtained from Carlo Erba, Milan, Italy. All other solvents were of analytical grade and they were used as supplied. Trifluoroacetic acid (TFA) and tetrabutylammonium chloride (TBACl) were obtained from Aldrich (Milwaukee, WI, USA).

2.2. Apparatus

A laboratory-made extraction device (Fig. 1) was prepared by pouring 0.5 g of CarboGraph 4 (surface area, 210 m²/g, 120–400 mesh size) (Carbochimica Romana, Rome, Italy) into a 6.5×1.3 cm I.D. medical grade polypropylene tube (Supelco) containing one polyethylene frit (Supelco) at the bottom. A second frit was placed gently on the sorbent bed to avoid crushing of the particles. The CarboGraph 4 cartridge was fitted into a side-arm filtration flask and liquids were forced to pass through the cartridge by vacuum (water pump). Before processing water samples, the cartridge was washed with 10 ml of the eluent phase for phenols (see below), followed by 2 ml methanol and 14 ml HCl-acidified water (pH 2).

2.3. Sampling

Grab samples of surface waters (5.7–11.3 mg/l DOC) were collected in brown bottles from various rivers flowing between Florence and Rome and kept at 4°C in the dark until analysis. Unless they contained unusually large amounts of suspended materials, river water samples could be extracted unfiltered (although with restricted flow-rates). When necessary, river waters were filtered through 1.5-μm pore size Whatman GF/C glass fiber-pads (Maidstone, UK),

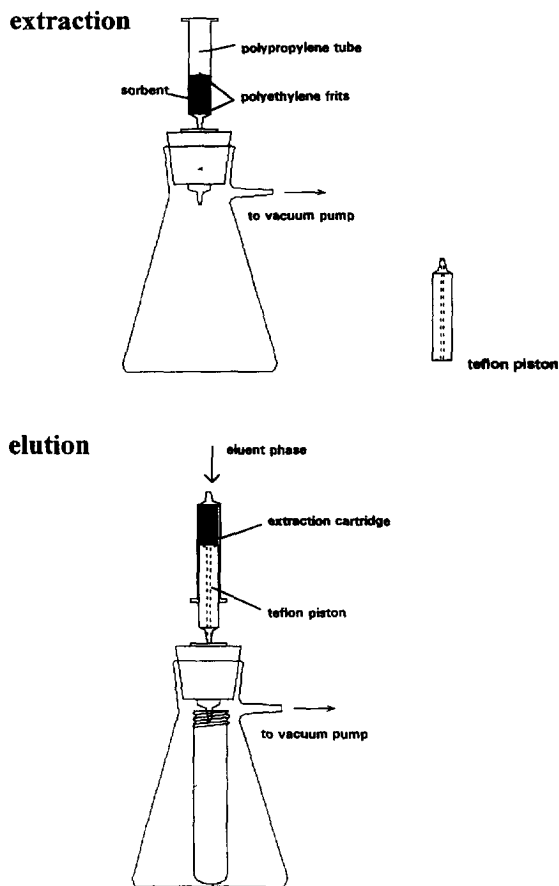


Fig. 1. Schematic view of the laboratory-made extraction device used in this work.

2.4. Procedure

For recovery studies, aqueous samples were fortified with known amounts of the working standard solution. Before spiking hypochlorite-containing water samples with phenols, 0.5 g/l Na₂S₂O₃·5H₂O was added to the sample to avoid oxidation of the analytes. Water samples were agitated for 1 min and, after 2 min, poured in a glass reservoir connected to the sorbent cartridge. Water was forced to pass through the cartridge at flow-rates of ca. 100 ml/min by reducing the pressure in the vacuum apparatus to the minimum. After the sample was passed through the column, the pump was disconnected and the cartridge was filled with 7 ml of distilled water,

which was allowed to pass through the cartridge at flow-rates of 5–7 ml/min. Any void space, created by some shrinking of the sorbent bed occurring during the passage of the sample, was eliminated by pushing the upper frit against the sorbent bed. Most water was removed from the cartridge by pulling air from the room through it for 1 min. The pump was disconnected, 0.8 ml of methanol was poured into the cartridge, which was slowly passed through the sorbent bed to eliminate residual water. Following the passage of methanol, the pressure was reduced to the minimum for 1 min. Thereafter, a suitably drilled cylindrical teflon piston with one conically indented base and a Luer tip was forced to enter the cartridge until it reached the upper frit. Then, the trap was turned upside down (Fig. 1), a 1.4-cm I.D. glass vial with a round bottom was placed below the cartridge and phenols were eluted by passing 6 ml of a methylene chloride–methanol (80:20, v/v) mixture containing TBACl, 10 mmol/l through the trap. The flow-rate at which the eluent phase was percolated through the cartridge was about 6 ml/min obtained by suitably regulating the vacuum in the apparatus. The last drops of this solvent mixture were collected by a further decrease in the pressure inside the flask. The extract was then divided in two equal portions which were dried in a water bath at 27°C under a gentle stream of nitrogen. The residue of one aliquot of the extract was reconstituted with 200 μ l of water/methanol (80:20, v/v) acidified with 0.6% (v/v) TFA. A 50- μ l sample of this solution was injected into the LC apparatus. For an eventual confirmation of phenols, the residue of the second extract was reconstituted with 150 μ l of water–acetonitrile (80:20, v/v) basified with Na₂CO₃, 0.1 mol/l, and the phenols were converted to acetyl derivatives by adding 40 μ l of acetic anhydride, and heating this solution to 50°C for 6 min. A 50- μ l sample of this solution was injected into the LC apparatus.

2.5. LC apparatus

A Model 5000 liquid chromatograph (Varian, Walnut Creek, CA, USA) equipped with a Rheodyne Model 7125 injector having a 50- μ l loop and with a

Table 1

Liquid chromatography retention times of eleven phenols and their corresponding acetyl derivatives

Compound	Retention time (min)	
	Intact	Acetyl derivative
Phenol	8.0	12.4
4-Nitrophenol	11.0	14.4
2-Chlorophenol	12.6	17.0
2,4-Dinitrophenol	13.2	16.2
2-Nitrophenol	14.0	13.8
2,4-Dimethylphenol	15.0	17.9
4-Chloro-3-methylphenol	15.9	19.9
2,4-Dichlorophenol	16.8	20.6
4,6-Dinitro-2-methylphenol	17.6	18.8
2,4,6-Trichlorophenol	19.8	23.6
Pentachlorophenol	24.4	28.4

Model 2550, programmable wavelength UV detector was used. For chromatographing phenols, an ‘‘Alltima’’ 25 cm \times 4.6 mm I.D. column filled with 5- μ m LC-18 reversed-phase packing (Alltech, Deerfield, IL, USA) was used. Pump A contained water acidified with 0.025% TFA and pump B contained acetonitrile acidified with 0.0125% TFA. Gradient elution was performed by increasing linearly the percentage of the organic modifier from 22% to 90% in 27 min. The flow-rate was 1.0 ml/min. The initial wavelength of 280 nm was changed to 220 nm after 18 min. Phenol derivatives were chromatographed under the same conditions with the difference that the wavelength was set constantly at 220 nm. Retention times of the eleven phenols and of their corresponding acetyl derivatives, measured under the chromatographic conditions reported above, are listed in Table 1.

The concentrations of phenols in water were calculated by comparing the areas of the peaks obtained with the sample and with the working standard solution. This standard was prepared by dissolving a given amount of the composite working standard solution into the solvent mixture used to desorb phenols from the GCB trap and carrying this solution through the final part of the procedure. Peak area measurements were carried out with the aid of a Model 1020 PE Nelson integrator (Perkin-Elmer, Norwalk, CT, USA).

3. Results and discussion

3.1. Analyte trace enrichment

Determination of polar target compounds present in water at the ng/l level needs extraction procedures that are capable of recovering analytes from large water volumes. The extraction efficiency of Carbo-graph 4 material was evaluated in comparison with Carbograph 1, another well-known example of GCB material used by us in the past. A drinking water sample was spiked with the working standard solution to obtain concentrations of 100–200 ng/l for each phenol. Then, 4-l fractions of the sample were analyzed by extraction with cartridges containing 0.5-g of the two GCBs, alternately. Measurements were made in duplicate. Results reported in Table 2 show that, although the surface area of Carbograph 4 is only about twice as large as that of Carbograph 1, Carbograph 4 is much more efficient in extracting phenol. This effect could be traced to the fact that, compared to Carbograph 1, the Carbograph 4 material possesses on its surface a larger number of geometrical and/or chemical heterogeneities which enable it to establish stronger interactions with adsorbates.

Re-extraction of phenols from the Carbograph 4

cartridge in the conventional way, that is by allowing the organic solvent mixture to flow through the sorbent bed in the same way as the water sample, resulted in low recoveries of the two dinitrophenols and PCP. These three phenols were eluted as very tailed peaks from the sorbent column, and when four additional 6-ml fractions of the eluent mixture were passed through the cartridge in succession, steadily decreasing amounts of the three phenols were found in each of the eluates. This problem was circumvented by turning the cartridge upside down and back-flushing it with the eluent system.

It is noteworthy that, even by reversing the Carbograph 4 cartridge, poor recovery of these three weakly acidic phenols was obtained by using 6 ml of a TFA-acidified solvent mixture. This eluent phase was found to be effective in removing them from the Carbograph 1 surface [19]. This effect can be explained because phenols interact more strongly with the surface of the Carbograph 4 and because an ion pair-forming agent is more effective than an acidic one in eluting acidic species from a GCB column rapidly [21].

Besides extraction of large water volumes, high enrichment factors can be reached by minimizing the final extract volume. However, even partial removal of the solvent is known to cause large and erratic

Table 2
Recovery of phenols on extracting 4 l of drinking water by cartridges containing 0.5 g of two different GCB sorbent materials

Compound	Recovery (%) ^a		
	Carbograph 1		Carbograph 4
	Forward elution	Forward elution	Back-flush elution
Phenol	26 ^b	95	96
4-Nitrophenol	93	65	99
2-Chlorophenol	53	97	98
2,4-Dinitrophenol	93	15	96
2-Nitrophenol	97	97	98
2,4-Dimethylphenol	63	98	100
4-Chloro-3-methylphenol	98	96	99
2,4-Dichlorophenol	96	98	98
4,6-Dinitro-2-methylphenol	97	10	95
2,4,6-Trichlorophenol	93	96	98
Pentachlorophenol	100	36	101

^a Mean values obtained from duplicate measurements.

^b Recoveries of phenols remained unaltered in the back-flushing mode.

losses of the analytes. These losses are strictly dependent on the conditions chosen for concentrating extracts [13]. By varying some experimental conditions, optimization studies of the solvent concentration step were performed. To simulate actual situations exactly, 100-ml fractions of LC grade water were carried through the extraction and elution procedures. Eluates were divided in two 3-ml portions, which were first spiked with known volumes of the working composite standard solution, and then taken to dryness. These additions were made to simulate analyses of drinking water samples containing 50–100 ng/l of each analyte. Four sets of experiments were conducted under various solvent removal conditions. For each set of conditions, six recovery experiments were conducted. Results are reported in Table 3. Complete recovery of the analytes was obtained by drying the extract at 27°C and taking the precaution of not allowing the residue-containing vial to stay in the water bath for more than a few minutes after the solvents appeared to be completely removed (experiment 1). When the vial containing the extract was left in the bath for 5 min after complete solvent removal, a significant loss of the most volatile phenols was observed (experiment 2). Similar results were obtained by drying the extract at 35°C (experiment 3). If TBACl was not

added to the solvent mixture, severe evaporative losses of most of the phenols were observed on drying the extract (experiment 4). Apparently, the presence of TBACl in the CH₂Cl₂–CH₃OH mixture makes the analytes less volatile. This beneficial effect could have been caused by the formation of non-volatile ion pairs occurring between quaternary ammonium ions and phenols. This assumption however relies on the possibility that even the least acidic phenols are present in the solvent mixture as phenoxy ions. The solvent evaporation step was performed at 27°C, and the vial was removed from the water-bath 1–2 min after the solvents were evaporated.

3.2. Accuracy and precision

The analytical recovery and the precision of this method were assessed. A municipal water sample was spiked with the eleven phenols to obtain individual concentrations of 50–100 ng/l. This sample was then divided in six 4-l aliquots for analysis. Four different river water samples were spiked with phenols at individual concentrations of 1–2 µg/l. From each of the four samples, two 1-l aliquots were taken and the resulting eight samples were analysed. Typical quantitative results, reported in Table 4,

Table 3
Recovery of phenols after solvent removal (spike level: 50–100 ng/l)

	Recovery ^a ±R.S.D. (%) ^b			
	Experiment 1 ^c	Experiment 2 ^d	Experiment 3 ^e	Experiment 4 ^f
Phenol	95±1.7	91±1.2	89±2.2	22±6.7
4-Nitrophenol	99±3.1	98±3.3	98±2.5	98±2.1
2-Chlorophenol	97±2.2	94±0.5	92±2.4	40±11.6
2,4-Dinitrophenol	98±2.5	97±1.7	96±1.4	86±4.7
2-Nitrophenol	95±2.0	87±1.5	85±3.6	57±7.1
2,4-Dimethylphenol	96±2.9	95±0.7	92±1.8	40±6.8
4-Chloro-3-methylphenol	96±1.3	94±0.8	92±1.5	66±6.6
2,4-Dichlorophenol	96±0.8	95±1.1	93±1.8	62±14.0
4,6-Dinitro-2-methylphenol	100±1.3	100±1.2	97±1.5	84±2.8
2,4,6-Trichlorophenol	97±2.4	94±0.8	94±1.3	69±1.5
Pentachlorophenol	98±1.4	99±1.2	98±1.4	98±(1.7)

^a Mean values obtained from six measurements.

^b R.S.D.= relative standard deviation.

^c Extract dried at 27°C.

^d After drying at 27°C, the residue was kept at this temperature for 5 min.

^e Extract dried at 35°C.

^f Extract dried at 27°C without TBACl.

Table 4
Recovery of phenols after extraction from 4 l of drinking water (spike level: 50–100 ng/l) and 1 l of river water (spike level: 1–2 $\mu\text{g/l}$)

Compound	Recovery \pm R.S.D.(%)	
	Drinking water ^a	river water ^b
Phenol	95 \pm 7.2	97 \pm 2.3
4-Nitrophenol	98 \pm 1.5	98 \pm 0.7
2-Chlorophenol	99 \pm 4.5	99 \pm 1.3
2,4-Dinitrophenol	96 \pm 1.1	98 \pm 1.8
2-Nitrophenol	91 \pm 1.3	97 \pm 2.1
2,4-Dimethylphenol	95 \pm 1.0	93 \pm 1.1
4-Chloro-3-methylphenol	95 \pm 1.1	98 \pm 3.5
2,4-Dichlorophenol	98 \pm 4.6	97 \pm 0.5
4,6-Dinitro-2-methylphenol	101 \pm 1.3	94 \pm 0.9
2,4,6-Trichlorophenol	96 \pm 2.3	99 \pm 1.9
Pentachlorophenol	98 \pm 5.4	94 \pm 0.7

^a Mean values obtained from six measurements.

^b Mean values obtained from eight measurements.

show that the extraction efficiency of the CarboGraph 4 cartridge was independent of the concentration of the phenols. This result demonstrated that adverse effects from irreversible adsorption by the materials composing the extraction apparatus were absent. It was also evident that the cartridge was not overloaded by fulvic acids and other unknown organic species present in surface water samples.

When a LC procedure making use of a detector such as the UV one, which is not sufficiently selective, is employed for monitoring very low levels of target compounds in natural waters, false positives or overestimations caused by unknown compounds may occur. To confirm the presence of phenols tentatively identified and to correctly measure their concentrations, an aliquot of the extract is re-injected into the same analytical LC column after conversion of phenols to their corresponding acetyl derivatives by acetic anhydride in a moderately basic medium. In this way, the presence of a certain phenolic compound in the sample can be confirmed by observing the disappearance from the chromatogram of the peak for the authentic phenolic species and the simultaneous appearance of the peak for its corresponding derivative. The optimum reaction conditions were evaluated by varying some parameters. Initial assays performed by extracting river water samples spiked with phenols showed that the presence of fulvic acids co-extracted with phenols did not affect the yield of the reaction. To simulate an actual situation, 100-ml aliquots of drinking water

samples were extracted as described above. Each eluate from the CarboGraph 4 cartridge was divided in two equal 3-ml portions. Before drying, the two portions were spiked alternately with known volumes of two distinct working standard solutions, one containing six phenols and the other solution containing the other five phenols considered. This subdivision was made in order to avoid the occurrence of some overlapping between peaks for authentic phenols and those for acetyl derivatives. Dried residues were invariably redissolved in a moderately basic solution (see Experimental section) and acetyl derivatives of phenols were formed by varying some parameters, such as temperature, time, and amount of the reagent. In all cases, derivatization experiments were carried out on 1 μg of each phenolic compound. Reaction yields were assessed by measuring peak areas produced by residual underivatized amounts of phenolic compounds. In all experiments, measurements were made in duplicate. Results are reported in Table 5. A satisfactory conversion of phenols to acetyl derivatives was observed in all experiments, except for the two dinitrophenols and 2,4-dimethylphenol. Among these three phenols, DNOC appeared to be the least prone to undergo attack by acetic anhydride. Under mild conditions, low reaction yields for the three phenols mentioned above can be explained on the basis of steric hindrance by both methyl and nitro groups. In addition, the two dinitrophenoxy ions are less nucleophilic than the more basic analogs. The best

Table 5

Reaction yields upon conversion of phenols to acetyl derivatives obtained under various conditions

Compound	Temperature (°C)							
	20				50			
	Time(min)							
	3		6		3		6	
	Reagent volume (μ l)							
	20		40		20		40	
	Reaction yield (%) ^a							
Phenol	80	88	89	94	87	94	95	98
4-Nitrophenol	87	91	96	99	96	99	98	100
2-Chlorophenol	86	93	97	98	97	100	98	100
2,4-Dinitrophenol	75	89	88	93	82	89	81	93
2-Nitrophenol	86	96	98	99	100	100	100	100
2,4-Dimethylphenol	66	72	82	85	83	90	90	94
4-Chloro-3-methylphenol	89	93	98	98	97	100	99	100
2,4-Dichlorophenol	90	93	91	95	97	98	100	100
4,6-Dinitro-2-methylphenol	41	53	51	55	56	71	58	78
2,4,6-Trichlorophenol	85	91	93	97	92	99	100	100
Pentachlorophenol	89	93	94	98	94	98	98	99

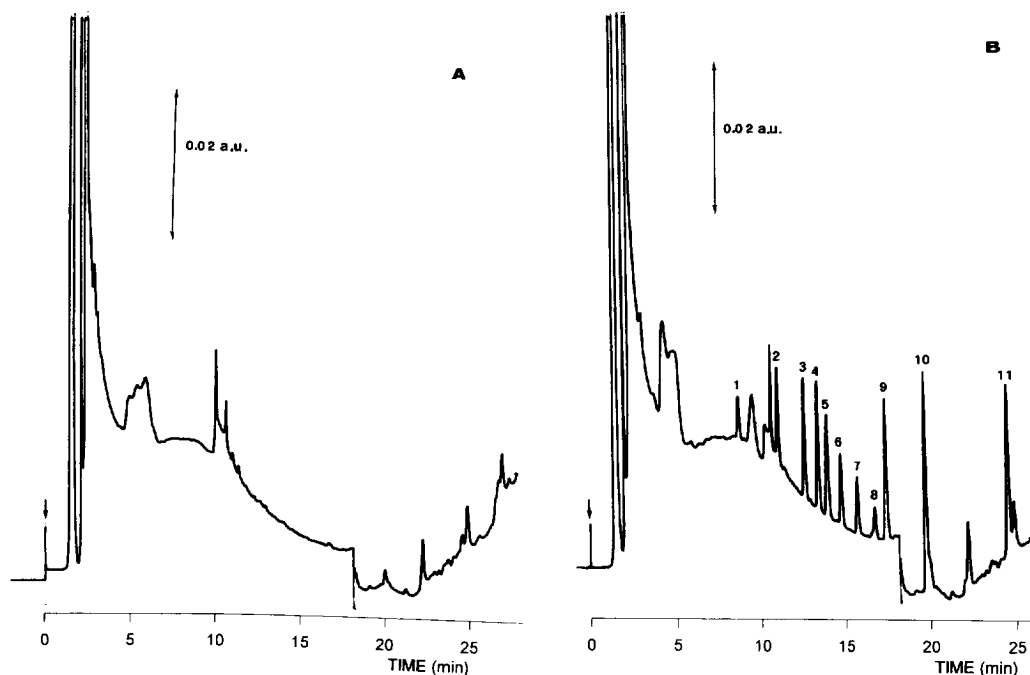
^a Mean values obtained from duplicate experiments.

Fig. 2. LC chromatograms obtained by analyzing 4 l of a drinking water sample unspiked (A) and spiked (B) with eleven phenols at the individual level of 50–100 ng/l. Compounds: 1=phenol; 2=4-nitrophenol; 3=2-chlorophenol; 4=2,4-dinitrophenol; 5=2-nitrophenol; 6=2,4-dimethylphenol; 7=4-chloro-3-methylphenol; 8=2,4-dichlorophenol; 9=4,6-dinitro-2-methylphenol; 10=2,4,6-trichlorophenol; 11=pentachlorophenol.

result was achieved by allowing DNOC react with 40 μl of the derivatizing agent for 6 min at 50°C. The reaction yield for DNOC did not significantly increase by increasing the reagent volume from 40 to 60 μl , while even lower reaction yields were obtained by increasing both the time and the temperature of reaction. Chromatograms obtained from the analysis of 4 l of a municipal water sample spiked with phenols at individual concentrations of 50–100 ng/l by this method are shown in Figs. 2 and 3.

As an example and to show the potential of this method in terms of selectivity, the chromatogram shown in Fig. 4A was obtained by analyzing 1 l of a river (Tiber) water sample. Interpretation of the

chromatogram based upon retention time measurement indicated that the sample could be contaminated by both 2-chlorophenol and 2,4-dinitrophenol at levels of 1.3 and 1.9 $\mu\text{g/l}$, respectively. To obtain confirmation of the presence in the sample of the two phenols tentatively identified, the second 3-ml aliquot of the same extract was first dried and then carried through the derivatization procedure. Fig. 4B shows the chromatogram generated by injection of a fraction of the reaction mixture into the LC column. After interpretation of this chromatogram, the presence in the Tiber water of 1.3 $\mu\text{g/l}$ of 2-chlorophenol was substantiated by the complete disappearance of the peak having the same retention time as

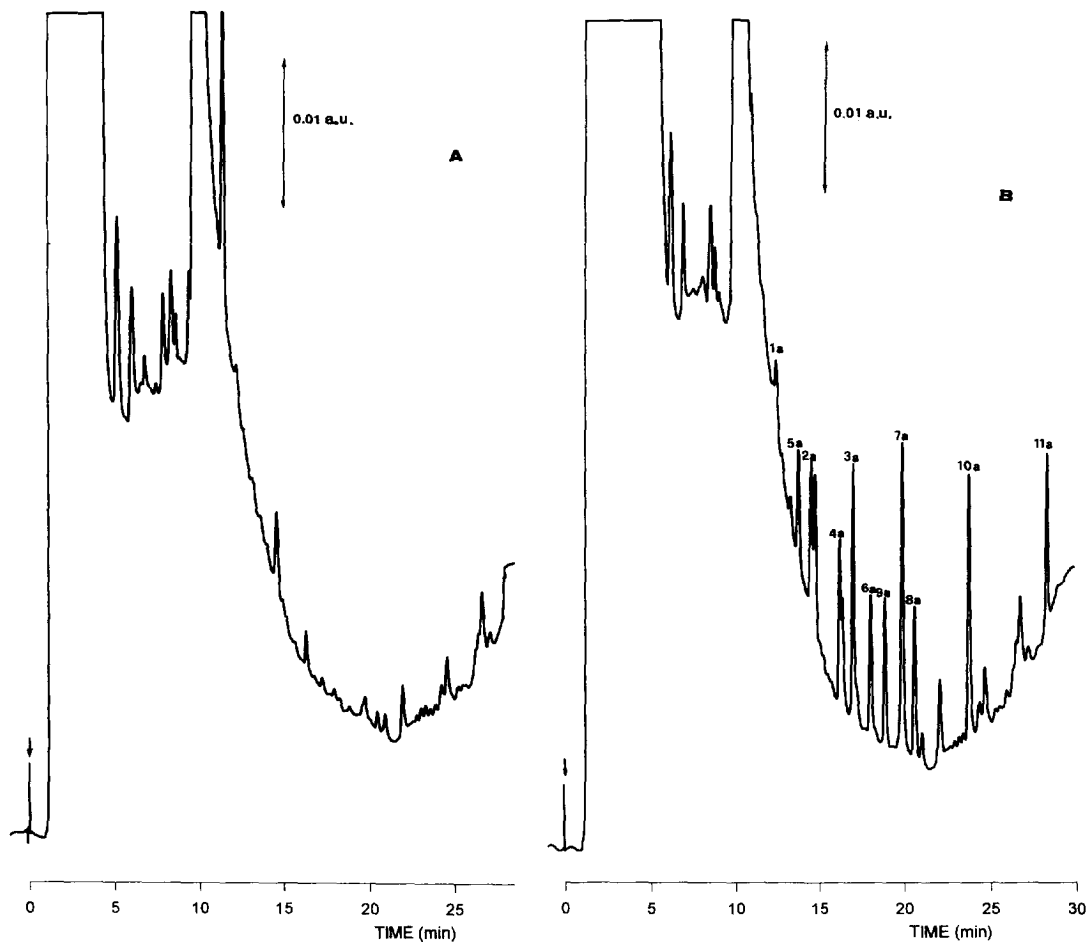


Fig. 3. LC chromatograms obtained by injecting extracts after addition of acetic anhydride and relative to analysis of 4 l of a drinking water sample unspiked (A) and spiked (B) with eleven phenols at the individual level of 50–100 ng/l. Peaks for acetyl derivatives of phenols are labelled as in Fig. 2 with the difference that the number is followed by the letter a.

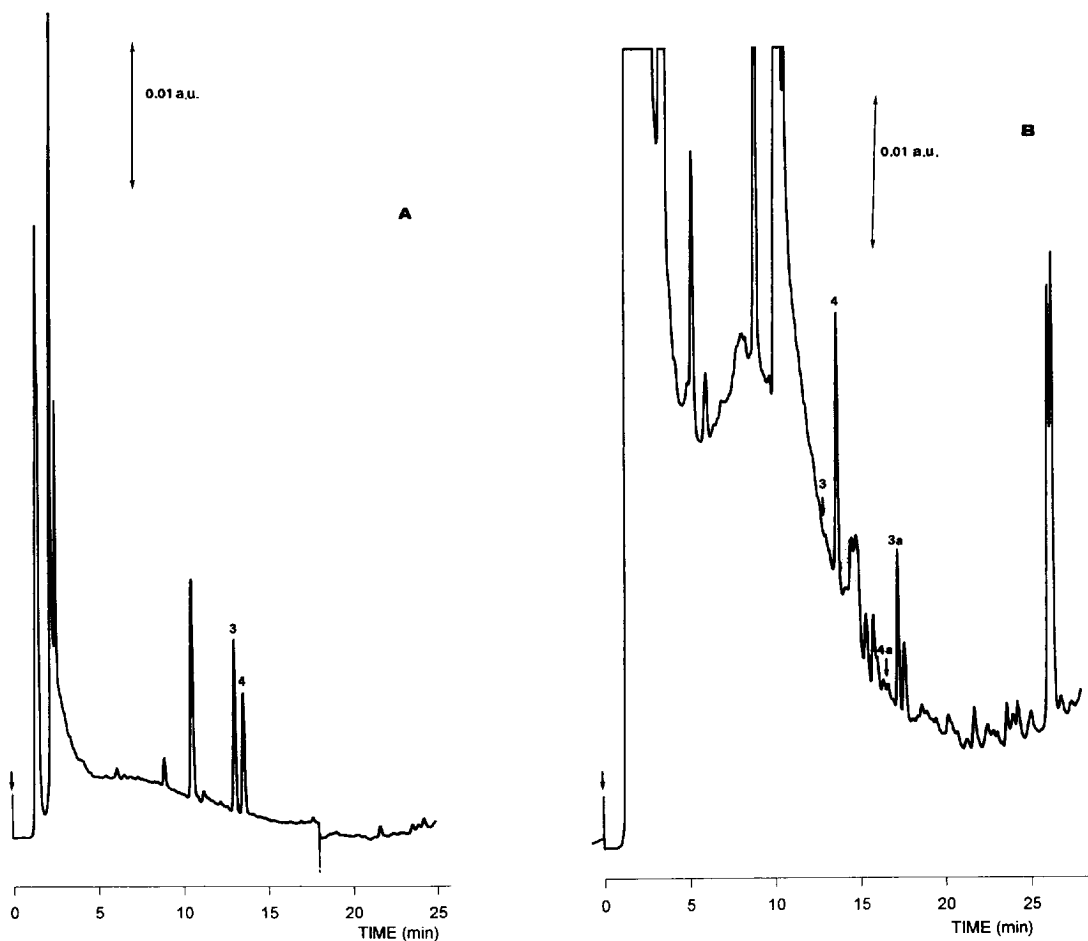


Fig. 4. LC chromatograms obtained by injecting extracts without (A) and with (B) addition of acetic anhydride and relative to analysis of 1 l of a river (Tiber) water sample. Peak numbering is the same as in Figs. 2 and 3. As measured by us, the concentration of 2-chlorophenol was 1.3 $\mu\text{g/l}$.

that for 2-chlorophenol and the simultaneous appearance of a peak having the same retention time as that for the acetyl derivative of 2-chlorophenol. For the opposite reason, the possibility that the peak having the same retention time as 2,4-dinitrophenol could be in part or totally produced by the presence of this phenolic compound in the river water was rejected. In fact, after addition of acetic anhydride, the peak having retention time as that for 2,4-dinitrophenol did not disappear either in part or totally and no new peak for the acetyl derivative of this phenolic compound was observed.

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