ELSEVIER

Journal of Chromatography A, 778 (1997) 301–311

JOURNAL OF CHROMATOGRAPHY A

Determination of priority phenolic compounds in water and industrial effluents by polymeric liquid–solid extraction cartridges using automated sample preparation with extraction columns and liquid chromatography
Use of liquid–solid extraction cartridges for stabilization of phenols

M. Castillo, D. Puig, D. Barceló*

Department of Environmental Chemistry, CID-CSIC, c/Jordi Girona 18–26, 08034 Barcelona, Spain

Abstract

Fourteen phenolic compounds: catechol, phenol, 4-nitrophenol, 4-methylphenol, 2,4-dinitrophenol, 2-nitrophenol, 2-chlorophenol, 4-chlorophenol, 3-chlorophenol, 4-chloro-3-methylphenol, 2,4-dimethylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol, which are included in the priority pollutants list of the US Environmental Protection Agency and in the European Union list 76/464, were determined in water by liquid–solid extraction (LSE) followed by liquid chromatography with UV detection (LC–UV). Three different polymeric sorbents were used: Isolute ENV+, Lichrolut EN and Porapak RDX. The developed method involves the use of automated sample preparation with extraction columns (ASPEC XL) for automated sample preconcentration and a Baker LSE 12G apparatus with the vacuum set at 15 p.s.i. (1 p.s.i.=6894.76 Pa) for the drying step. The recoveries varied from 70 to 100% (except for catechol) on preconcentrating 700–1000 ml of a 5 μg/l solution, pH 2.5–3. The stability of the target compounds on Isolute ENV+ was evaluated by storing the sorbent material at −20°C, 4°C and at room temperature for up to three months. Complete recovery was observed after storage at −20°C for two months. At room temperature, losses of up to 70% were observed for phenol, catechol and the more volatile phenols. The stability of the phenolic compounds was dependent on the water matrix, the storage temperature and on physico chemical properties, such as vapor pressure and water solubility. © 1997 Elsevier Science B.V.

Keywords: Water analysis; Stability studies; Adsorbents; Sample handling; Environmental analysis; Phenols; Nitrophenols; Chlorophenols

1. Introduction

Phenolic compounds are chemical substances that are present in many industrial processes and, as a consequence, they are released in many industrial effluents and waste water. Due to their toxicity and persistence in the environment, a variety of phenols were included in different monitoring programs, such as those of the US Environmental Protection Agency (EPA) and of the European Union (EU).

Determination of phenolic compounds in water, in the range of 0.1 to 0.4 μg/l is especially difficult, as reported in a few inter-laboratory studies [1]. The extraction and recovery of all phenolic compounds

*Corresponding author.

PII S0021-9673(97)00313-0

present difficulties, due to their wide range of polarities and the relatively high vapor pressure that enhances losses by volatilization. As a consequence, there is a need to develop more efficient extraction and stability methods for phenolic compounds from water matrices.

Regarding the isolation of organic pollutants from water, the US EPA methodology for the analysis of priority phenols in water samples involves sample acidification to pH 2 and dichloromethane liquid–liquid extraction (LLE) [2,3]. However, LLE has many drawbacks and there is a general trend to use liquid–solid extraction (LSE) protocols instead of the current LLE procedures [4].

LSE materials, such as cartridges, Empore disks or packed in small precolumns for on-line procedures were used in the preconcentration of phenols from water samples. C_{18}-based sorbents were not suitable for the simultaneous preconcentration of polar and non-polar compounds, due to low breakthrough volume (V_B) of the more polar analytes. Breakthrough for catechol, phenol and 4-nitrophenol occurred when loading a 50-ml water sample on 500 mg of C_{18} sorbent [5]. Other sorbents, such as C_{18}-OH (with a specific area of approximately 400 m^2/g) and the polymeric sorbents that are based on styrene–divinylbenzene, such as Amberlite XAD-2 or XAD-4 [6,7] or PRP1 [8], improved the results obtained with C_{18} (110 m^2/g of specific area) even though the preconcentration of polar compounds was not completed [9]. The use of polymeric phases with a high cross-linking grade and specific area (LiChrolut EN, Isolute ENV+, Porapak RDX, Amberchrom, Envi-chrom, PLRP-S) should be able to solve the enrichment problem, as reported for a variety of analytes [6,7,10,11].

Analysis of the phenolic extract is usually performed by gas chromatography (GC) of derivatized samples, but derivatization of phenols is not straightforward [11]. Recently, the EPA has reported a new protocol (method 8041) [12], which recommends the derivatization of phenols to methylated phenols instead of to pentafluorobenzoyl ether derivatives. However, this method requires the use of diazomethane, which is carcinogenic and explosive, and the potential hazards associated with its use are well known. Liquid chromatography (LC) is a good alternative to GC and it overcomes the above-mentioned limitations. The absence of derivatization requirements and the possibility of on-line coupling with LSE has made LC a very applied separation technique for the analysis of phenolic compounds using C_{18} or C_8 columns (in most cases) and UV detection at 280 and 310 nm (for nitrophenols and pentachlorophenol) [9,10,13]. Diode array detectors [14] and electrochemical detection [15] have also been used. In the last few years, the coupling of LC with mass spectrometry (MS) became a good alternative to classical LC detectors, especially with the advantage of atmospheric pressure (API) LC–MS interfaces [16].

The EPA's recommended method for the stabilization of phenolic compounds in water (using a glass container and water that was acidified to pH 3 and stored at 4°C) led to 15% losses for some phenolic compounds [9]. Referring to the stability study, it is remarkable the absence of experiments done with these compounds preconcentrated in polystyrene–divinylbenzene polymeric sorbents. Previous studies were carried out in our laboratory for a variety of pesticides and sorbent materials [17–19]. In this way, safe transport and storage of water samples could be achieved. Other studies showed that the stability of organic pollutants stored in LSE discs were, in general, better than those obtained when storage was carried out in a water matrix [20,17].

The objectives of this paper were: (i) To compare the behaviour of three different sorbents (Isolute ENV+, LiChrolut EN and Porapak RDX) for the preconcentration of a variety of phenolic compounds from water samples, (ii) to apply the developed method to the determination of phenolic compounds in waste water effluents, with LC–MS confirmatory analysis and (iii) to perform stability studies under three different storage conditions (room temperature, 4°C and −20°C) in one polymeric cartridge material. To our knowledge, such a study has not been undertaken previously for this group of pollutants and using the methodology presented in this paper.

2. Experimental

2.1. Chemicals

HPLC-grade water, methanol and acetonitrile were

obtained from Merck (Darmstadt, Germany) and were passed through a 0.45-μm membrane filter before use. Phenolic compounds were purchased from Merck, except for catechol and phenol, which were from Sigma (St. Louis, MO, USA) and 3-chlorophenol, which was from Aldrich (Milwaukee, WI, USA).

2.2. Liquid chromatography–UV detection

Experiments were performed using an automated sampler processor from Gilson (Villiers-le-Bel, France). This system includes one automatic sampler processor, model ASPEC XL, equipped with two Rheodyne six-port valves, one high-pressure preconcentration pump, model 305, one low-pressure pump, model 401C, one eight-port valve, model 817, valve actuator. The HPLC system was purchased from Gilson and consists of two pumps (model 305), one 811c dynamic mixing chamber, an 805 manometric module and a UV detector, model 117.

Gradient elution was performed using acetonitrile–water, both acidified with 1% acetic acid, with the following gradient: from acetonitrile–water (30:70, v/v) in isocratic mode over 15 min to 100% acetonitrile over 15 min and back to the initial conditions in 5 min, at a flow-rate of 1 ml/min. A Hypersil Green ENV column (150×4.6 mm I.D.), equipped with a guard column, was used (both were from Shandon Hypersil, Cheshire, UK).

2.3. LC atmospheric pressure chemical ionization (APCI)–MS conditions

A VG Platform from Fisons Instruments (Manchester, UK), equipped with a standard atmospheric pressure ionization (API) source that can be configured as APCI or ion spray ionization (ISP) was used. The APCI interface consists of a heated nebulizer probe and the standard atmospheric pressure source was equipped with a corona discharge pin. For APCI experiments, the source and probe temperatures were set at 150 and 400°C, respectively. The corona discharge voltage was maintained between 2 and 3 kV and the cone voltage was optimized between 10 and 70 V. The counter electrode high voltage (HV) lens voltage was set at 0.18 kV. The chromatographic conditions were the same as those reported above for LC–UV. In full scan mode, the scan range was from 90 to 400 m/z in negative ion (NI) mode.

2.4. Sample preparation

Automated sample preparation with extraction columns (ASPEC XL) was used. The system was fitted with an external 306 LC pump for the dispensing of samples through the LSE cartridges and with an 817 switching valve, for the preconcentration of water samples.

Ground water samples (pH=8.0, 75 mg/l nitrate, 387 mg/l sulfate, 254 mg/l Ca, 88 mg/l Mg; conductivity, 2020 μs/cm) were spiked to give a final concentration of 5 μg/l of each phenolic compound and these were acidified to pH 2.5–3 immediately before extraction. Preconcentration was performed using the ASPEC XL system by means of disposable 6 ml cartridge columns packed with 200 mg of LiChrolut EN from Merck, with 200 mg of Isolute ENV+ from International Sorbent Technology (Cambridge, UK) and with 500 mg of Porapak RDX from Waters (Milford, MA, USA). All sorbents were based on polystyrene–divinylbenzene polymers. Conditioning of the sorbents was accomplished by passing 7 ml of methanol and 3 ml of water through the cartridges at a flow-rate of 1 ml/min. The sorbent was not allowed to dry and different volumes (300, 500, 700, 1000 and 1500 ml) of a spiked water sample were loaded. The drying step was carried out using a Baker LSE 12g apparatus connected to a vacuum system with pressure set at 15 p.s.i. (1 p.s.i.=6894.76 Pa) (negative pressure). Drying took 20–30 min. The elution step was performed by adding 2×5 ml of acetonitrile to the cartridge and waiting 5 min between the two aliquots in order to keep a good contact time between the solvent and the trapped compound. The final evaporation step of the extra solvent was carried out with a stream of nitrogen, which evaporated approximately 17 μl of the extract per minute. The extracts were concentrated to a final volume of 0.5 ml. Special care was needed in the evaporation step in order to avoid losses of the more volatile compounds. Finally, the extract was diluted with 0.5 ml of HPLC-grade water and 20 μl were injected into the LC system.

2.5. Quantitation

External calibration was used for quantitation. A series of injections of the target compounds (2.5, 5, 10, 20 and 30 ng) were used to obtain the calibration graphs and to determine the calibration equations, which were linear over the studied range (see Table 1). The limits of detection (LODs) were determined by injecting sample extracts that were serially diluted until the signal-to-noise ratio (S/N) for any single analyte reached a value of three. The limits of quantification (LOQs) were calculated from LODs by applying a factor of 3.3. Repeatability studies were performed ($n=5$) to establish the relative standard deviation (R.S.D.) of the method.

2.6. Stability study

Although the recovery study was performed using LiChrolut EN, Isolute ENV+ and Porapak RDX sorbents, the stability study of the phenolic compounds was carried out using only Isolute ENV+. This was because both LiChrolut EN and Isolute ENV+ showed similar efficiency for the preconcentration of target compounds in water samples up to 500 ml, according to the R.S.D. of the method. In contrast, Porapak RDX turned out to be unsuitable for the preconcentration of phenols (see Table 2) and, therefore, was excluded from the stability study. After conditioning, preconcentration of a 500-ml ground water sample spiked to 5 μg/l with each phenolic compound and drying 36 cartridges of Isolute ENV+, the columns were wrapped in aluminium foil and stored at −20°C, 4°C and room temperature (RT) for a period of three months. After 0.5, 1, 2 and 3 months, the cartridges were thawed for a period varying from 1–2 to 6–8 h, depending on the storage conditions and, afterwards, they were analyzed using the protocol described above.

3. Results and discussion

3.1. Recovery studies

Various elution volumes (2×3, 2×4 and 2×5 ml) of methanol and acetonitrile were tested after preconcentrating the water sample containing phenolic compounds. Acetonitrile enhanced the recovery of all compounds, except catechol, which was more easily eluted with methanol, due to hydrogen bond interactions.

The evaporation step also needed to be optimized, due to the high volatility of some of the target compounds (e.g. phenol or catechol), which would lower their recoveries. Four different methods for concentrating the extract were tested: (i) Applying a

Table 1
Calibration equations (2.5 to 30 ng), LODs and LOQs for all target compounds

Compound	Wavelength (nm)	Equation[a]	r^2	LOD (μg/l)	LOQ (μg/l)
Catechol	280	67 153.7+20 107.2W	0.99341	0.25	0.83
Phenol	280	−60 721.5+50 817.7W	0.99512	0.2	0.67
4-Nitrophenol	310	132 913.0+79 250.7W	0.99725	0.03	0.10
4-Methylphenol	280	8150.1+24 733.1W	0.99943	0.15	0.50
2-Chlorophenol	280	−62 751.1+72 176.7W	0.99942	0.05	0.17
2,4-Dinitrophenol	310	−44 298.9+47 901.5W	0.99940	0.04	0.13
2-Nitrophenol	280	−15 988.0+56 714.4W	0.99809	0.025	0.08
4-Chlorophenol	280	3233.5+25 039.1W	0.99811	0.1	0.33
3-Chlorophenol	280	−14 660.1+12 747.9W	0.99854	0.9	3.00
2,4-Dimethylphenol	280	645.4+49 589.8W	0.99959	0.1	0.33
4-Chloro-3-methylphenol	280	−11 565.1+26 142.2W	0.99845	0.15	0.50
2,4-Dichlorophenol	280	−25 421.9+65 627.8W	0.99759	0.08	0.27
2,4,6-Trichlorophenol	280	−3292.8+866 605W	0.99905	0.6	2.00
Pentachlorophenol	310	−4423.1+8540.5W	0.99952	0.8	2.67

[a] W=injected mass (ng).

Table 2
Mean recoveries (%) and R.S.D.s ($n=5$) obtained on loading different sample volumes spiked to 5 μg/l with each phenolic compound on LiChrolut EN, Isolute ENV+ and Porapak RDX

Compound	Sample volume (ml)								
	Isolute ENV+			Lichrolut EN			Porapak RDX		
	500	1000	1500	500	1000	1500	500	1000	1500
Catechol	28 (13)	0	0	28 (11)	0	0	17 (11)	0	0
Phenol	64 (12)	59 (4)	33 (9)	79 (15)	79 (7)	37 (13)	51 (9)	40 (9)	14 (12)
4-Nitrophenol	75 (15)	74 (5)	68 (12)	89 (8)	87 (13)	45 (11)	55 (13)	35 (10)	25 (10)
4-Methylphenol	65 (12)	60 (10)	50 (10)	93 (9)	91 (9)	38 (9)	40 (14)	26 (11)	18 (13)
2,4-Dinitrophenol	72 (13)	73 (10)	29 (11)	79 (15)	82 (10)	42 (10)	48 (10)	41 (11)	22 (9)
2-Nitrophenol	69 (14)	64 (10)	37 (9)	80 (13)	83 (7)	44 (11)	54 (11)	39 (12)	21 (10)
2-Chlorophenol	79 (16)	60 (12)	54 (13)	84 (10)	84 (8)	41 (8)	38 (10)	29 (9)	21 (11)
4-Chlorophenol	70 (9)	60 (8)	60 (7)	86 (13)	89 (9)	41 (12)	50 (8)	35 (13)	25 (12)
3-Chlorophenol	62 (13)	64 (12)	62 (11)	86 (12)	93 (15)	43 (10)	52 (9)	33 (13)	27 (9)
2,4-Dimethylphenol	64 (11)	55 (10)	53 (13)	76 (10)	81 (11)	37 (11)	36 (8)	34 (10)	19 (11)
4-Chloro-3-methylphenol	72 (9)	65 (8)	61 (8)	86 (13)	86 (10)	42 (13)	50 (10)	37 (11)	27 (10)
2,4-Dichlorophenol	67 (12)	62 (9)	59 (10)	80 (10)	86 (8)	42 (9)	53 (14)	44 (13)	25 (14)
2,4,6-Trichlorophenol	85 (10)	84 (11)	68 (12)	104 (13)	92 (7)	51 (9)	60 (10)	55 (10)	50 (10)
Pentachlorophenol	81 (4)	65 (10)	24 (11)	89 (9)	87 (13)	46 (12)	47 (11)	40 (9)	23 (11)

gentle stream of nitrogen, which caused the evaporation of either 37 μl/min, (ii) or 17 μl/min of solvent, (iii) rotary evaporation and (iv) the addition of 80 μl of a 0.6-*M* methanolic solution of NaOH followed by the application of a stream of nitrogen to allow solvent evaporation to occur [13]. Methods (i) and (iv) were ruled out, as low recoveries were obtained for all target compounds (method i) and catechol was lost completely (method iv). Methods (ii) and (iii) yielded similar results, however, method (ii) was chosen because it allowed the simultaneous evaporation of twelve samples, using the LSE 12 g apparatus, and did not lead to contamination of the samples.

The results obtained using the optimized LSE method for the preconcentration of phenolic compounds in three different polymeric sorbents are shown in Table 2. In all cases, breakthrough occurred for catechol when volumes of sample greater than 300 ml were used. This fact can be attributed to the high polarity and affinity for water, as shown in the physico chemical properties reported in Table 3. Even though mononitrophenols have a lower affinity for the sorbent than dinitrophenols, slightly better recoveries were obtained for mononitrophenols.

There was no great difference between the results obtained using Isolute ENV+ and LiChrolut EN, based on the R.S.D. of the method, for the preconcentration of up to 500 ml water samples. Better recoveries were obtained with LiChrolut EN at higher sample volumes. With regard to the sorbent Porapak RDX, the results showed that it is not suitable for the preconcentration of phenolic compounds, as breakthrough occurs on loading 300 ml of water. It was not possible to determine the reason for the different behaviours of the three sorbents, as the necessary manufacturer's data about the sorbents' characteristics was not available. Nevertheless, the similar specific surface areas for Isolute ENV+ and LiChrolut EN (1100 and 1200 m^2/g) could explain the similar recoveries obtained using both of these sorbents.

Fig. 1 shows a comparative chromatogram between the preconcentration of 700 ml of ground water spiked with 5 μg/l of the phenolic mixture in the three different polymeric sorbents.

Determination and quantification of all phenolic compounds was possible by preconcentrating 500 ml of ground water spiked to 5 μg/l in both Isolute ENV+ and LiChrolut EN sorbents, leading to 80–90% recovery for most of the analytes, whereas only 28% of the catechol was recovered. When it is not necessary to determine catechol, the optimum load-

Table 3
Physicochemical properties of target compounds ([28–30])

Compound	log K_{ow} ([29,30])	Vapor pressure (mm Hg) ([28])	pK_a ([28,29])
Catechol	NA	40 (118°C)	NA
		760 (245°C)	
Phenol	1.46	1 (40°C)	9.9
		760 (181°C)	
4-Nitrophenol	2.04 (29)	NA	7.08
	1.90 (30)		
4-Methylphenol	1.94	1 (53°C)	NA
		760 (201°C)	
2-Chlorophenol	2.15	NA	8.56
2,4-Dinitrophenol	1.67 (29)	1 (49°C)	3.94
	1.53 (30)		
2-Nitrophenol	1.89 (29)	1 (12°C)	7.23
	1.78 (30)	760 (210°C)	
4-Chlorophenol	2.39	1 (49°C)	9.2 (28)
		760 (220°C)	9.38 (29)
3-Chlorophenol	2.50	1 (44°C)	NA
		760 (214°C)	
2,4-Dimethylphenol	2.42	NA	10.4
4-Chloro-3-methylphenol	3.10	NA	9.6
2,4-Dichlorophenol	3.23	1 (56°C)	7.6 (28)
		760 (210°C)	7.85 (29)
2,4,6-Trichlorophenol	3.72 (29)	1 (76°C)	6.15
	3.69 (30)	10 (120°C)	
Pentachlorophenol	5.24 (29)	40 (200°C)	4.9 (28)
	5.01 (30)	760 (300°C)	4.75 (29)

NA=Not available.
k_{ow}=octanol–water partition coefficient. 1 mm Hg=133.322 Pa.

ing volume in LiChrolut EN was 1 l, which led to the same recovery as that reported for 500 ml.

3.2. Environmental waters

The developed method was applied to the preconcentration of river water samples. Fig. 2 illustrates the extraction of river water under neutral and acidic conditions. Even though catechol and phenol were better detected under neutral conditions, the rest of the compounds suffered important losses under these conditions. For example, nitro-derivatives and pentachlorophenol were completely lost when the extraction was performed under neutral conditions. Consequently, further experiments were performed with extraction being performed under acidic conditions.

The LODs of the target compounds extracted in natural waters are higher than those obtained in HPLC-grade or potable waters, as expected [21]. The ensuing method allowed the determination of 4-chloro-3-methylphenol, 3-chlorophenol and 2,4,6-trichlorophenol at the 0.1 μg/l level, catechol at the 0.25 μg/l level and the rest of the compounds could be quantified at concentrations lower than 0.1 μg/l, which conformed with the determination level required by current EU legislation [22]. Fig. 3 shows the chromatograms obtained on preconcentration of 1 and 0.5 l of ground water, spiked to 0.1 μg/l and 0.5 μg/l with each phenolic compound, respectively.

3.3. Waste water

The method was used to determine priority phenols in an industrial effluent from a pulp industry. The pulp industry utilizes large amounts and a wide variety of chemicals, as it uses various chemical processes and discharges large volumes of effluents. In some cases, the pulp is brightened or bleached by a variety of chemical techniques, which give rise to

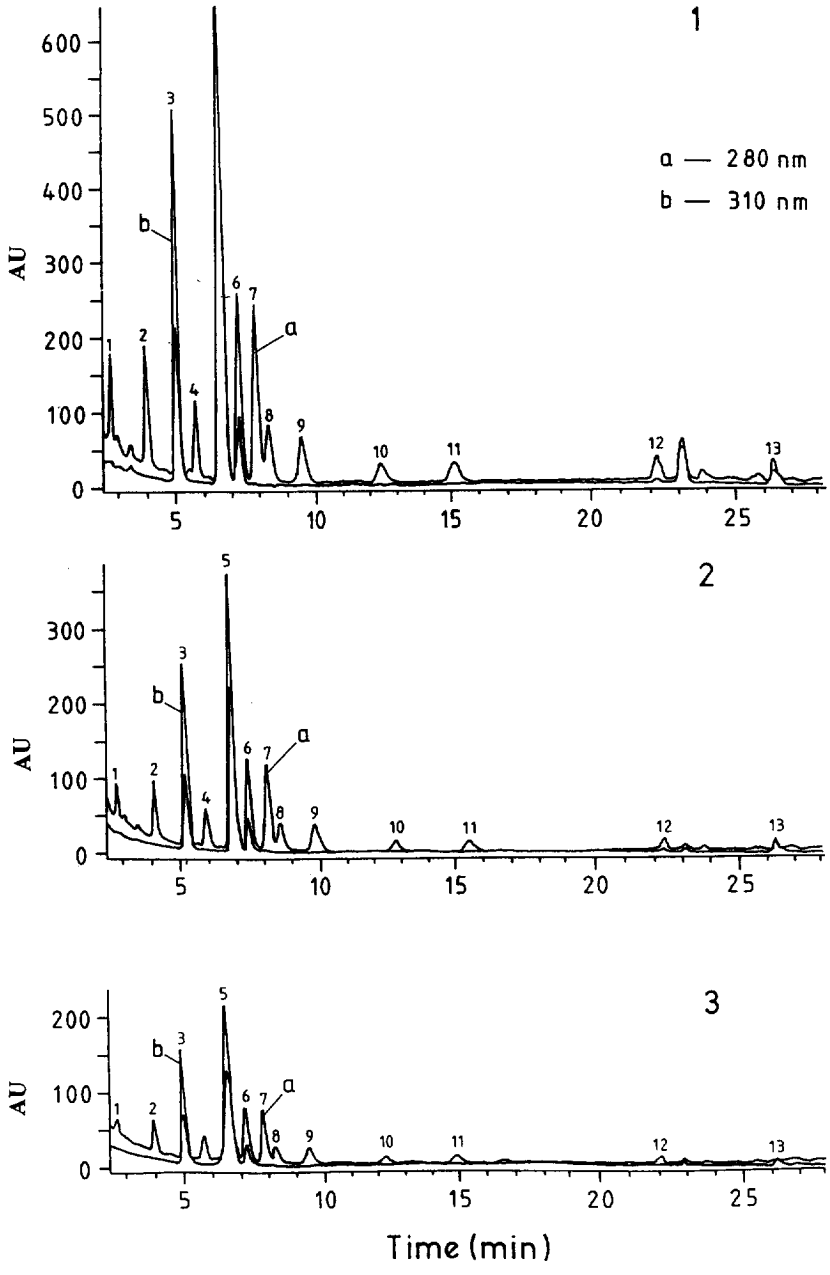

Fig. 1. LC–UV chromatograms obtained after preconcentration of 700 ml of ground water spiked to a final concentration of 5 μg/l: 1=catechol, 2=phenol, 3=4-nitrophenol, 4=4-methylphenol, 5=2,4-dinitrophenol, 6=2-nitrophenol, 7=4-chlorophenol, 8=3-chlorophenol, 9=2,4-dimethylphenol, 10=4-chloro-3-methylphenol, 11=2,4-dichlorophenol, 12=2,4,6-trichlorophenol and 13=pentachlorophenol. Three different sorbents were tested: (1) LiChrolut EN, (2) Isolute ENV+ and (3) Porapak RDX. For LC conditions, see Section 2.

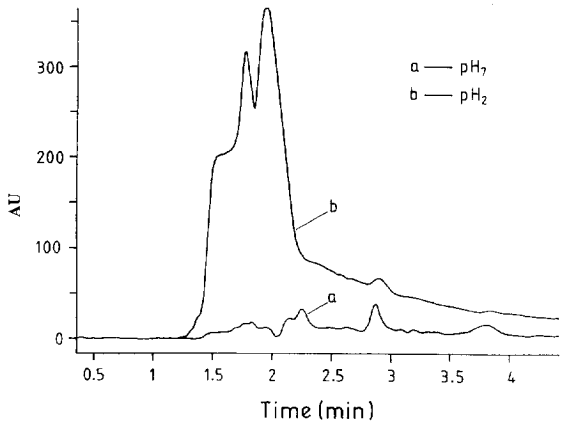

Fig. 2. Interferences extracted in the preconcentration of river water under neutral and acidic conditions. A 500-ml volume of river water, spiked to 5 μg/l with the above-mentioned phenolic compounds (see Fig. 1) were loaded on LiChrolut EN. For LC conditions, see Section 2.

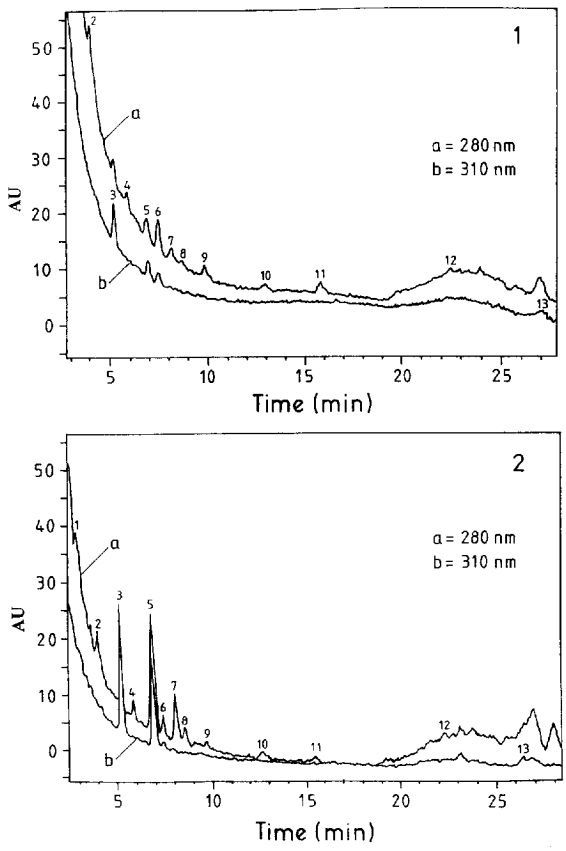

Fig. 3. LC–UV chromatograms obtained in the determination of the LOD of the method. (1) Preconcentration of 1000 ml of ground water spiked to 0.1 μg/l with each phenolic compound on LiChrolut EN. (2) Preconcentration of 500 ml of ground water spiked to 0.5 μg/l with each phenolic compound on LiChrolut EN. All target compounds are detected on preconcentrating 1 l of ground water spiked to 0.1 μg/l with each phenolic compound on a LiChrolut EN cartridge, except for catechol. For LC conditions, see Section 2.

the discharge of a number of contaminants such as chlorocatechols and chlorophenols. The only effluent treatments that are widely used by the pulp industry involve primary treatment, consisting of elimination of suspended solids and secondary treatment with microbiological oxidation of fermentable dissolved organic constituents [23]. However, there is evidence that organic compounds, such as chlorophenols or chlorocatechols, are not completely eliminated by this process, as was pointed out by the EPA [24]. Analysis of the effluents before and after treatment was carried out using the methodology developed in this work. LC–APCI-MS was used to confirm the data obtained from UV detection.

The cone voltage was set at 35 V because in previous research it was found to be a good compromise between sensitivity and structural information, as can be seen in Table 4, where the main ions are shown for selected phenolic compounds.

The results are summarized in Table 4 and are in the range of those found in other similar industries [25]. Data from LC–APCI-MS in full scan mode showed the presence of 2-chlorophenol, 2,4-dichlorophenol, 4-nitrophenol and catechol, but no traces of highly chlorinated phenols and of other priority phenols were found. The values obtained from untreated water varied from 2.5 to 27.3 μg/l. However, even though these levels were reduced by the treatment, complete removal did not occur. Surprisingly, catechol was also found in this case, but not in the original effluent, which indicates that it was formed during the water treatment process. This should be attributed to the very complex nature of the pulp industry effluents, which usually contain a number of organic contaminants, such as resin and fatty acids, a variety of chlorinated organic compounds, such as chlorophenols, chlorocatechols or guaiacols, and volatile compounds (including the group designated as total reduced sulfur compounds), such as methylmercaptans or methyldisulfides. Tetra-

Table 4
Concentrations (μg/l) and main ions of priority phenols found in a pulp effluent before and after treatment by LC–APCI-MS in negative ion mode

Compound	M_r	Before treatment	After treatment	Main ions
4-Nitrophenol	139	2.5	2.3	$[M-H]^-$ (*m/z*: 138) $[M-H-NO]^-$ (*m/z*: 108) $[M-H-NO_2]^-$ (*m/z*: 92)
2-Chlorophenol	128	27.5	18	$[M-H]^-$ (*m/z*: 127)
2,4-Dichlorophenol	162	17.5	13	$[M-H]^-$ (*m/z*: 161) $[M-HCl]^-$ (*m/z*: 126)
Catechol	110	–	4	$[M-H]^-$ (*m/z*: 109)

chlorocatechols and tetrachloroguaiacols were reported to be present in pulp effluents in the range of 2 to 240 and 1 to 120 μg/l, respectively, depending on the type and plant design and on the applied water treatment processing step [25].

3.4. Stability study

The National Pesticide Survey (NPS)–EPA requires that all monitored pesticides included in their programmes should be stable in water for at least fourteen days, after being inhibited biologically with 10 ml of $HgCl_2$ (or monochloroacetic buffer or sulfuric acid at pH<3) and being stored at 4°C [26]. On applying this criteria to phenolic compounds, it was found that 2,4-dimethylphenol, 3-chlorophenol, trichlorophenols, pentachlorophenol, 2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol (10 μg/l), which were present in river water acidified with H_2SO_4 to pH 3 and which was stored in dark glass at 4°C, were stable for 28 days whereas other phenolic compounds, such as phenol, catechol, 2-chlorophenol, 4-chlorophenol and 4-methylphenol suffered 15% losses [9]. Regarding these results, it was found necessary to develop new methods for stabilizing phenolic compounds and for improving their transport and storage characteristics.

Triplicate samples were analyzed for each storage time and temperature. Isolute ENV+ cartridges were preconcentrated with 500 ml of a water sample and stored at −20°C, 4°C and at RT for a period of up to three months. Analysis were performed after 0.5, 1, 2 and 3 months of storage. Losses were similar for all phenolic compounds, except for catechol, which was the more unstable. Table 5 shows the results obtained after different storage times at RT, 4°C and −20°C, respectively.

Losses at RT were quite important for all target compounds, even after short periods of storage. Consequently, the transport of phenolic compounds retained in LSE cartridges has to be carried out under refrigeration conditions. For instance, 60% of catechol was lost after storage at RT for the first fifteen days, whereas 25% of the remaining phenols were lost under the same conditions. Nevertheless, it is possible to transport water samples spiked with phenolic compounds and preconcentrated in LSE polymeric cartridges stored at 4°C, as is shown in these results. No losses were observed when storage took place at 4°C over the first fifteen days, enabling samples to be shipped. Under these conditions, the results again showed that catechol was the most unstable phenolic compound (50% losses after one month of storage) and 2,4-dinitrophenol was the most stable (20% losses after storage for one month) whereas the remaining phenolic compounds suffered losses in the range of 25–35%. With regard to storage at −20°C, it is remarkable that no losses were observed after one month, losses of up to 18% were obtained after two months, except for 2,4-dinitrophenol and 2-nitrophenol, which suffered losses of 12%, which were of the same order as the R.S.D. of the method. Storage at −20°C is the most reliable method for storing phenolic compounds in polymeric LSE sorbents, as also reported in other solid-phase extraction methods for pesticides [17,18].

It is important to take into account that the stability of phenols is dependent on various parameters, such as the water matrix, temperature and physicochemical properties, e.g. vapor pressure and

Table 5
Losses (%) suffered after different storage times at −20°C, 4°C and RT. A 300-ml volume of ground water spiked to 5 μg/l with each phenolic compound was loaded on an Isolute ENV+ cartridge

Compound	Storage conditions											
	RT				4°C				−20°C			
	Storage time (months)				Storage time (months)				Storage time (months)			
	0.5	1	2	3	0.5	1	2	3	0.5	1	2	3
Catechol	23	50	55	70	15	23	55	70	0	0	20	39
Phenol	20	20	37	43	10	20	37	43	0	0	26	33
4-Nitrophenol	17	39	24	51	5	17	24	51	0	0	18	30
4-Methylphenol	16	20	31	44	10	16	31	44	0	0	22	30
2,4-Dinitrophenol	10	23	18	36	7	10	18	36	0	0	13	26
2-Nitrophenol	14	22	17	38	10	14	17	38	0	0	10	27
2-Chlorophenol	13	25	21	47	9	13	21	47	0	0	14	32
4-Chlorophenol	17	19	29	50	13	17	29	50	0	0	23	38
3-Chlorophenol	15	17	30	52	8	15	30	52	0	0	19	37
2,4-Dimethylphenol	11	20	24	49	6	11	24	49	0	0	17	35
4-Chloro-3-methylphenol	15	24	28	55	11	15	28	55	0	0	20	34
2,4-Dichlorophenol	15	18	26	57	8	15	26	57	0	0	21	35
2,4,6-Trichlorophenol	18	21	33	63	10	18	33	63	0	0	24	38
Pentachlorophenol	20	23	30	57	12	20	30	57	0	0	21	37

water solubility [27]. The vapor pressure of target compounds explained their losses, which were due mainly to their volatilization. Those compounds with poor affinity for the sorbent and high vapor pressure suffered maximum losses, as was shown for catechol. The best results were obtained for those compounds that are highly retained, probably due to their $\pi-\pi$ interactions with the sorbent material, e.g. in the case of 2,4-dinitrophenol.

4. Conclusions

A semi-automated method for the determination of phenolic compounds in natural waters has been developed. Preconcentration of ground water was achieved using LiChrolut EN and Isolute ENV+ as the LSE sorbents. The determination of phenol, 4-nitrophenol, 4-methylphenol, 2,4-dinitrophenol, 2-nitrophenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol at 0.1 and 5 μg/l levels was carried out using a 1-l water sample. For the determination of catechol at 0.25 μg/l, it was necessary to load 0.5 l of ground water in both Isolute ENV+ and LiChrolut EN sorbents. It is remarkable that it was possible to detect phenol at 0.2 μg/l.

The V_B values in Isolute ENV+ and LiChrolut EN were higher than 1 l for all target compounds, except for catechol, which had a V_B that was lower than 300 ml.

Porapak RDX was not suitable for the preconcentration of phenolic compounds in natural waters as V_B occurred for percolation of water samples lower than 300 ml for all target compounds.

Phenolic compounds were stabilized in LSE material. The great advantage of using disposable LSE cartridges for stabilizing phenols in water samples is the storage space, since cartridges replace 1 l bottles. Complete recovery is observed after storage for two months at −20°C and after 0.5 months at 4°C for all target compounds. This indicates that it is possible to ship the LSE cartridges, containing phenolic compounds under refrigeration conditions for a period of up to fifteen days from the sampling site to the central laboratory for the final analysis, making it unnecessary to perform the analysis immediately after sampling. Degradation in LSE cartridges was attributed to volatility and poor affinity for the sorbents of the most polar compounds.

Acknowledgments

This work was supported by the projects 'Protocol for the Evaluation of Residues in Industrial Contaminated Liquid Effluents Program (Pericles)' (Contract ENV4-CT95-0021) from the Environment and Climate Program, 1994–1998, Commission of the European Communities and CICYT (AMB96-1675-CE). We thank Merck and IST for supplying the LSE cartridges.

References

[1] Aquacheck, Summary of the Results from Samples Distributed in March and July 1994, WRC, Medmenham, UK, 1994.
[2] EPA method 604. Phenols in Federal Register, Friday October 26, 1984, Environmental Protection Agency, Washington, DC, Part VIII, 40 CFR, Part 136, pp.58–66.
[3] EPA method 625. Base/Neutrals and Acids in Federal Register, Friday October 26, 1984, Environmental Protection Agency, Washington, DC, Part VIII, 40 CFR, Part 136, pp. 153–174.
[4] D. Puig and D. Barceló, Trends Anal. Chem., 15 (1996) 362–375.
[5] M.T. Galcerán and O. Jáuregui, Anal. Chim. Acta, 304 (1995) 75–84.
[6] C.D. Chriswell, R.C. Charjand and J.S. Fritz, Anal. Chem., 47 (1975) 1325.
[7] A. Takeda and J.S. Fritz, J. Chromatogr., 152 (1978) 329.
[8] C.E. Werkhoven-Goewie, W.M. Boon, A.J.J. Prat, R.W. Frei, U.A.Th. Brinkman and C.J. Little, Chromatographia, 16 (1982) 53.
[9] D. Puig and D. Barceló, Chromatographia, 40 (1995) 435–445.
[10] P.J.M. Kwakman, D.A. Kamminga, U.A.Th. Brinkman and G.J. de Jong, J. Chromatogr., 553 (1991) 345.
[11] P. Mubmann, K. Levsen and W. Radeck, Fresenius' J. Anal. Chem., 348 (1994) 654.
[12] EPA Method 8041, Phenols by Gas Chromatography: Capillary Column Technique, Environmental Protection Agency, Washington, DC, 1995, pp. 1–28.
[13] A.Di Corcia, S. Marchese, R. Samperi, G. Cecchini and L. Cirilli, J. Assoc. Off. Anal. Chem. Int., 77 (1994) 446.
[14] E. Pocorull, G. Sanchez, F. Borrull and R.M. Marcé, J. Chromatogr, 696 (1995) 31.
[15] D. Puig and D. Barceló, Anal. Chim. Acta, 311 (1995) 63.
[16] D. Puig, I. Silgoner, M. Grasserbauer and D. Barceló, J. Am. Soc. Mass Spectrom., 31 (1996) 1297–1307.
[17] S. Lacorte, N. Ehresmann and D. Barceló, Environ. Sci. Technol., 29 (1995) 2834–2841.
[18] E. Martínez and D. Barceló, Chromatographia, 42 (1996) 72–76.
[19] C. Molina, P. Grasso, E. Benfenati and D. Barceló, J. Chromatogr. A, 737 (1996) 47–58.
[20] D. Barceló, S. Chiron, S. Lacorte, E. Martínez, J.S. Salau and M.C. Hennion, Trends Anal. Chem., 13 (1994) 352–360.
[21] S. Chiron, A. Fernández-Alba and D. Barceló, Environ. Sci. Technol., 27 (1993) 2355–2356.
[22] M.C. Hennion, V. Pichon and D. Barceló, Trends Anal. Chem., 13 (1994) 361.
[23] C.C. Walden, D.J. McLeay and A.B. McKague, in The Handbook of Environmental Chemistry, Vol. 3, Part D, Cellulose Production Processes, Springer-Verlag, Berlin, pp. 1–28.
[24] W. Dellinger, in Development of a Document for Effluent Limitations, Guidelines and Standards for Pulp, Paper and Paperboard and the Builder's Paper and Board Mills, EPA Publ. No. 440/1-80/025-b, US Environmental Protection Agency, Washington, DC, 1980.
[25] NCASI: Experience with the Analysis of EPA's Organic Priority Pollutants and Compound Characteristics of Pulp Mill Effluents, Tech. Bull. No. 343, National Protection Agency, Washington, DC, 1980.
[26] I. Ferrer and D. Barceló, J. Chromatogr. A, 737 (1996) 93.
[27] J.H. Montgomery and L.M. Welkom, Groundwater Chemicals Desk Reference, Lewis, Chelsea, MI, 1990.
[28] R.C. West (Editor), Handbook of Chemistry and Physics, CRC Press, Cleveland, OH, 56th ed., 1975–1976.
[29] B.I. Escher and R.P. Schwarzenbach, Environ. Sci. Technol., 30 (1996) 263.
[30] J.R. Dean, W.R. Tomlinson, V. Makovskaya, R. Cumming, M. Hetheridge and M. Comber, Anal. Chem., 68 (1996) 131.