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Development of an automated on-line solid-phase extraction–high-performance liquid chromatographic method for the analysis of aniline, phenol, caffeine and various selected substituted aniline and phenol compounds in aqueous matrices

J. Patsias, E. Papadopoulou-Mourkidou*

Pesticides Science Laboratory, Aristotle University of Thessaloniki, P.O. Box 1678, 54006 Thessaloniki, Greece

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Abstract

A fully automated solid-phase extraction (SPE)–high-performance liquid chromatographic method has been developed for the simultaneous analysis of substituted anilines and phenols in aqueous matrices at the low- to sub-μg/l level. Diode array and electrochemical detection operated in tandem mode were used for analyte detection. Two new polymeric sorbent materials (Hysphere-GP and Hysphere-SH) were evaluated for the on-line SPE of substituted anilines and phenols from aqueous matrices and their performance was compared with the PRP-1 and PLRP-S sorbents. Hysphere-GP sorbent packed in 10×2 mm cartridges was found to give better results in terms of sensitivity and selectivity of the overall analytical method. The proposed analytical method was validated for the analysis of these compounds in Axios river water that receives industrial, communal and agricultural wastes. The detection limits for all the compounds range between 0.05 and 0.2 μg/l, except for aniline and phenol which have detection limits of 0.5 and 1 μg/l, respectively (aniline detected by electrochemical detection). The recoveries for all the compounds are higher than 75% except for aniline (6%), phenol (50%) and 3-chlorophenol (67%). Finally, in order to evaluate the efficiency of the Hysphere-GP (10×2 mm) cartridges for sample stabilization and storage, the stability of the compounds of interest at the sorbed state onto these cartridges has been evaluated under three different temperature regimes (deep freeze, refrigeration, 20°C).

Keywords: Automation; Solid-phase extraction; Water analysis; Environmental analysis; Anilines; Phenols; Alkylphenols; Chlorophenols; Nitrophenols

1. Introduction

Anilines and phenols are involved in many industrial processes and frequently are released into the environment through industrial discharges. Moreover nitrophenols and chlorophenols occur in the environment as degradation products of the organophosphorus and chlorinated phenoxyalkanoic acid pesticides, respectively. Anilines also occur in the environment as degradation products of the phenylurea and dinitroaniline herbicides. Phenols are persistent in the environment and toxic at the low μg/l

*Corresponding author. Tel./fax: +30-31-471-478.

E-mail address: mourkidu@agro.auth.gr (E. Papadopoulou-Mourkidou).

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level [1]. In the 80/778/EEC directive of the European Union it is stated that the maximum admissible concentration for each individual phenol in drinking water should not exceed 0.1 μg/l [2]. Anilines are also of toxicological importance and the monitoring of their levels in environmental waters is important for the protection of health and the environment [3].

The most common techniques for the analysis of anilines and phenols in environmental waters are gas (GC) and high-performance liquid chromatography (HPLC) [1]. The direct analysis of phenols by GC is difficult [4] and GC analysis is usually performed after a derivatization step [1,5–7]. Anilines are also thermolabile and polar compounds and a derivatization step is often required before GC analysis [3,8–11]. Most of the derivatization processes however are not straightforward and sometimes require handling of hazardous chemicals. Since in HPLC analysis there are no derivatization requirements, it appears to be a good alternative to GC analysis and nowadays has been widely accepted as the method of choice [1,12].

The most widely used detection methods in HPLC analysis of anilines and phenols are UV (especially diode array) and electrochemical detection [1]. Mass spectrometric [13] and direct [14] or indirect [15–17] fluorescence detection has also been reported. UV detectors provide very good signal stability and in the case of diode-array detectors they can be used for analyte tentative confirmation purposes using UV spectra libraries. Electrochemical detectors are more sensitive than the UV detectors, however their performance is highly dependent on the type of samples analyzed. Components from dirty samples are deposited on the electrochemical cell and the detector sensitivity is rapidly decreased [18–20].

For the extraction of anilines and phenols from environmental aqueous samples both classical liquid–liquid partition (LLP) and solid-phase extraction (SPE) protocols have been employed. SPE is becoming more attractive nowadays and a number of different sorbents have been used until now either in the on-line or in the off-line mode [1,4,18,21–26]. Even though better detection limits for the polar compounds can be achieved with the off-line approach there is a general tendency nowadays to turn to fully automated systems based on the on-line SPE [1,12]. Silica-based (like C_{18}, C_{18}-OH and C_8) and styrene–divinylbenzene polymeric sorbents (like PRP-1 and PLRP-S) were the first sorbents to have been evaluated [24–26]. These sorbents provide very good chromatographic performance when coupled on-line with HPLC but suffer from early breakthrough of the more polar compounds. To overcome this problem new polymeric sorbents with high specific surface area (like LiChrolut EN, Isolute ENV, ENVI-Chrom P, BondElut ENV, etc.) have been introduced recently for the SPE of polar compounds from aqueous samples [21–23]. These sorbents provide high recoveries even for the most polar compounds, but several analyte band broadening occurs when coupled on-line with HPLC analysis [22]. This is mainly due to the high adsorption power of the analytes onto the sorbent, which makes analyte desorption difficult, especially with the weak mobile phase elution power under initial conditions.

The objective of this study was the development of an analytical method for the simultaneous analysis of anilines and phenols in aqueous matrices at the sub-μg/l level based on the on-line coupling of SPE and HPLC analysis. Aniline, phenol, caffeine and 25 substituted anilines and phenols were used as model compounds. Diode-array detection and electrochemical detection operated in tandem mode were selected for analyte detection. Two new polymeric sorbents (Hysphere-GP and Hysphere-SH) were evaluated and their performance was compared with PRP-1 and PLRP-S sorbents. In order to improve the peak shape of the detected analytes, the influence of cartridge diameter on the elution profile was investigated. The stability of the compounds under study at the sorbed state onto the Hysphere-GP (10×2 mm) cartridges was also investigated under three different temperature regimes (−24°C, ~4°C and 20°C), in order to explore the possibility for the stabilization and storage of aqueous samples onto these cartridges.

2. Experimental

2.1. Reagents and solvents

Reference materials for aniline, phenol, caffeine and for the various substituted anilines and phenols were purchased from Riedel-de Haen (Seelze-

Hanover, Germany). Stock standard solutions were prepared at a concentration of 1 mg/ml in methanol and were kept under deep freeze conditions (−24°C). Mixed working standard solutions were prepared at a concentration of 50 μg/ml by adding 2.5 ml of each stock standard solution to a 50-ml volumetric flask and diluting to 50 ml with methanol. With further dilutions standard solutions at concentrations of 25, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 μg/ml were prepared. These working standard solutions were kept also under deep freeze conditions and were renewed every 2 months.

Solvents (methanol and acetonitrile) were of HPLC grade purchased from Merck (Darmstadt, Germany). The water used as mobile phase component was laboratory-distilled water filtered through a 0.2-μm membrane filter (Gelman Science, Ann Arbor, MI, USA).

Potassium dihydrogenphosphate and orthophosphoric acid used for the preparation of the mobile phase buffer were purchased from Riedel-de Haen and Carlo Erba (Milan, Italy), respectively.

2.2. Chromatographic materials

PRP-1 (10×3 mm), PLRP-S (10×2 mm), Hysphere-GP (10×3 mm), Hysphere-GP (10×2 mm) and Hysphere-SH (10×3 mm) SPE cartridges were purchased from Spark Holland (Emmen, The Netherlands).

2.3. Instrumentation

The HPLC system consisted of the Model 222D pump and the Model 232C gradient controller (SSI, State College, PA, USA). For the injection of standard solutions the Basic Marathon autosampler (Spark Holland) equipped with a 20-μl injection loop was employed. The detection system consisted of the Model 996 diode-array detector (Waters, Milford, MA, USA) equipped with a 10-μl flow sell in series with the Decade electrochemical detector (Antec, Leyden, The Netherlands) equipped with a glassy carbon working electrode. The signal acquired from both detectors was recorded by a personal computer operated under the Millennium software (Waters).

The SPE of the aqueous samples was performed using the automated PROSPEKT system (Spark Holland).

2.4. Operational parameters

The HPLC analysis was performed on a Nucleosil 100-5 C_{18}, 150×4.6 mm, 5 μm reversed-phase column (Macherey-Nagel, Duren, Germany) thermostated at 30°C. The mobile phase was a binary gradient mixture of 5 m*M* phosphate buffer at pH 3 (solvent A) and a mixture of acetonitrile–HPLC water (90:10) (solvent B). The gradient composition and the flow-rate settings are shown in Table 1.

The diode-array detector was operated in the range of 190–400 nm with an acquisition rate of 2 spectra/s. After data acquisition qualitative and quantitative measurements were made at 215, 250 and 280 nm. The electrochemical detector was operated in the DC mode at +1.2 V. The reference electrode was filled, according to the manufacturers, with saturated LiCl because the mobile phase contained a high percentage of organic modifier.

Aqueous samples were filtered through a 0.2-μm membrane filter (Gelman Science) and acidified to pH 3 with orthophosphoric acid before loading. Before sample loading the SPE cartridges were conditioned with 10 ml methanol followed by 10 ml HPLC water (pH 3). After sample loading the SPE cartridges were washed with 2 ml HPLC water (pH 3) and eluted by the mobile phase in the backflush mode. The flow-rate during the conditioning and sample loading steps was 5 ml/min except for the Hysphere-GP (10×2 mm) cartridges when a 2 ml/min flow-rate was selected.

The analytical system was fully automated and could be operated unattended.

Table 1
HPLC gradient regime

Time (min)	A (%)	Flow (ml/min)
0	95	1.0
03	80	1.0
35	0	1.0
40	0	1.4
45	95	1.2
50	95	1.0

3. Results and discussion

3.1. Method development

3.1.1. HPLC analysis

A 5 m*M* phosphate buffer (pH 3)–acetonitrile mixture was used as mobile phase in order to improve the chromatographic behavior of the acidic phenolic compounds on the Nucleosil C_{18} column, because their retention is highly pH dependent. The phosphate buffer was selected among other mobile phase additives used for this purpose (such as acetic acid) in order to allow for the acquisition of spectral data up to the low UV range (190 nm). The low pH of the mobile phase has not been seen to affect the chromatographic performance for the relatively basic anilinic compounds.

3.1.2. Electrochemical detection

The electrochemical detector was found to be very sensitive for the studied anilines and phenols, but under continuous operation the sensitivity of the detector was continuously decreased (especially when analyzing dirty samples). Frequent cleaning of the electrochemical cell was therefore absolutely necessary to recover the initial sensitivity level. To improve the performance of the detector the working electrode was switched to the pulse mode for 2 min (between +1 V and −1 V for 1 s) before the beginning of each run with the mobile phase running at the initial conditions. The situation was improved but still there was an instability to the sensitivity. The linear regression coefficients (r^2) of the analytes ranged between 0.99 and 0.999. Daily recalibration of the detector was therefore absolutely necessary. The only compound for which the detector performed satisfactorily was aniline; the r^2 value was better than 0.999 and no daily recalibration was necessary for this compound. The response of the detector was also decreased gradually during runtime as the percentage of the organic modifier was increased. Even though the electrochemical detector proved to be very selective for the studied anilines and phenols, still there were interferences from the matrix and data from this detector were used only to provide additional evidence for the identity of the analytes detected by the diode-array detector.

3.1.3. Sample SPE

For the SPE of substituted anilines and phenols from aqueous matrices PRP-1, PLRP-S, Hysphere-GP and Hysphere-SH sorbents were evaluated.

Among the criteria used for the selection of the SPE cartridge were the recovery of the compounds of interest and the chromatographic performance (peak shape, peak width and asymmetry factor) of the overall chromatographic system for these compounds in order to achieve good selectivity and detection limits at the low- to sub-μg/l range.

Data of mean % analyte recovery vs. sample volume for 19 compounds covering the whole range of polarity were constructed for all the SPE cartridges under evaluation by sequentially extracting various sample volumes from 5 to 150 ml of an HPLC water sample spiked at the level of 2 μg/l. The acquired data are shown in Table 2 and plots for selected compounds are shown in Fig. 1. The acquired chromatograms when using the Hysphere-SH (10×3 mm), Hysphere-GP (10×3 mm) and Hysphere-GP (10×2 mm) SPE cartridges and 50 ml sample volume are shown in Fig. 2.

The PRP-1 (10×3 mm) and PLRP-S (10×2 mm) cartridges gave very good chromatographic peaks for all the compounds of interest, however the recovery of the very polar compounds was very poor (Table 2 and Fig. 1) resulting in very low detection limits for these compounds.

The Hysphere-SH (10×3 mm) and Hysphere-GP (10×3 mm) cartridges gave very good recoveries for the polar compounds (except for aniline), however the peak shape of these compounds was very bad (Fig. 2a and b) resulting again in poor sensitivity and selectivity. This was due to the high retention of the analytes onto those SPE sorbents. Similar behavior is reported in the literature for the LiChrolut EN material [22].

To investigate the influence of cartridge diameter onto the chromatographic performance, the Hysphere-GP sorbent was also tested in 2 mm I.D. cartridges. Because of the better refocusing of the sample onto the analytical column, the chromatographic performance was improved resulting in good selectivity and sensitivity; only phenol showed a

Table 2
Mean recoveries (n=3) vs. sample volume for the PRP-1 (10×3 mm), PLRP-S (10×2 mm), Hysphere-GP (10×2 mm) [Hy-GP(2)], Hysphere-GP (10×3 mm) [Hy-GP(3)] and Hysphere-SH (10×3 mm) [Hy-SH(3)] cartridges[a,b]

Analyte	Cartridge	Recovery (%)							
		Sample volume (ml)							
		5	10	25	50	75	100	125	150
Aniline	PRP-1	12.22	5.74	2.21	1.44	1.28	0.97	0.77	0.64
	PLRP-S	13.79	7.59	2.86	1.46	0.96	0.73	0.57	0.47
	Hy-GP(2)	55.00	27.63	10.92	5.63	3.75	2.56	2.11	1.77
	Hy-GP(3)	83.54	49.11	20.67	10.81	7.35	5.63	4.21	3.86
	Hy-SH(3)	72.77	48.84	29.36	16.09	10.47	8.44	6.46	5.53
Caffeine	PRP-1	99.9	94.8	57.5	28.5	19.4	14.2	11.3	9.7
	PLRP-S	100.0	94.9	60.7	30.6	19.5	14.5	12.0	10.2
	Hy-GP(2)	100.8	101.7	101.6	102.9	102.7	102.8	101.6	99.9
	Hy-GP(3)	100.6	100.7	101.8	102.1	101.3	102.4	101.2	101.9
	Hy-SH(3)	101.6	101.8	102.3	101.7	102.7	102.6	101.6	102.3
Phenol	PRP-1	29.4	54.1	20.5	10.5	7.2	4.9	3.9	3.5
	PLRP-S	17.3	50.6	23.1	11.2	7.8	5.1	4.2	3.7
	Hy-GP(2)	19.7	60.7	88.8	64.7	43.6	32.7	26.4	21.8
	Hy-GP(3)	38.9	58.4	99.7	99.8	93.3	82.8	65.8	55.1
	Hy-SH(3)	37.6	57.7	98.9	99.2	99.3	95.8	87.7	73.6
4-Nitroaniline	PRP-1	96.9	99.7	84.8	65.8	49.1	36.7	29.1	24.6
	PLRP-S	96.3	99.6	100.4	77.8	51.7	39.1	31.1	24.9
	Hy-GP(2)	97.3	101.3	100.8	100.4	101.4	101.7	100.5	100.2
	Hy-GP(3)	95.7	100.7	101.2	100.7	99.7	99.9	101.9	99.1
	Hy-SH(3)	95.5	98.2	98.0	90.1	79.6	70.3	63.8	53.0
4-Nitrophenol	PRP-1	95.2	99.9	76.4	53.9	35.9	26.9	21.6	17.9
	PLRP-S	95.5	99.3	85.0	58.7	38.4	29.3	23.7	19.5
	Hy-GP(2)	92.1	100.0	101.0	101.3	100.8	100.9	100.8	101.9
	Hy-GP(3)	90.4	97.0	101.9	100.5	100.9	101.1	101.9	102.1
	Hy-SH(3)	89.4	97.3	101.6	102.5	101.1	101.3	102.5	102.8
2-Chlorophenol	PRP-1	97.3	98.2	98.7	98.3	86.1	71.4	60.2	50.4
	PLRP-S	96.2	100.4	100.3	100.0	87.1	77.0	65.0	54.1
	Hy-GP(2)	59.8	82.5	99.2	101.5	101.8	100.8	100.4	100.4
	Hy-GP(3)	74.1	89.0	102.4	102.2	99.5	101.0	101.9	101.6
	Hy-SH(3)	55.7	75.9	99.4	100.0	101.7	100.4	100.4	99.5
2-Nitrophenol	PRP-1	88.2	98.9	99.9	100.4	100.1	101.2	100.5	101.4
	PLRP-S	89.4	99.0	100.2	100.1	99.8	100.4	99.7	100.3
	Hy-GP(2)	89.3	100.5	99.2	101.0	101.7	101.4	101.1	101.2
	Hy-GP(3)	82.8	92.5	98.7	101.2	99.0	99.5	98.3	99.6
	Hy-SH(3)	82.4	87.0	100.1	101.9	100.9	100.0	102.4	101.8
2,4-Dimethylphenol	PRP-1	77.0	101.8	102.3	102.1	101.7	101.7	99.5	101.6
	PLRP-S	81.0	90.5	98.4	100.1	102.8	102.6	101.0	101.8
	Hy-GP(2)	85.2	102.2	99.9	100.1	101.1	102.0	101.3	101.7
	Hy-GP(3)	88.8	102.8	97.0	100.8	97.6	99.1	100.5	101.6
	Hy-SH(3)	87.0	102.3	101.2	101.7	102.6	101.7	102.6	102.1
4-Chloro-3-methylphenol	PRP-1	90.7	101.6	99.4	101.8	101.2	102.0	102.5	102.2
	PLRP-S	90.4	97.9	101.1	102.2	105.4	105.3	106.7	105.1
	Hy-GP(2)	80.6	90.9	102.4	102.1	101.9	102.0	102.2	101.6
	Hy-GP(3)	82.9	91.9	99.2	101.0	101.6	102.4	101.6	102.2
	Hy-SH(3)	81.6	96.5	99.9	101.6	101.2	101.7	102.1	102.1
2,5-Dichlorophenol	PRP-1	92.2	100.2	102.0	100.1	105.4	101.2	102.3	102.3
	PLRP-S	92.1	101.1	101.7	100.0	101.2	102.6	101.4	102.5
	Hy-GP(2)	73.6	90.7	100.4	98.9	99.8	101.4	101.4	101.2
	Hy-GP(3)	76.1	87.2	96.2	99.6	101.6	101.2	101.1	102.1
	Hy-SH(3)	82.1	90.3	95.0	99.9	101.7	100.8	102.5	101.8

Table 2. Continued

Analyte	Cartridge	Recovery (%)							
		Sample volume (ml)							
		5	10	25	50	75	100	125	150
2,3-Dichloroaniline	PRP-1	93.6	100.1	98.4	102.0	102.0	101.6	102.9	101.8
	PLRP-S	94.5	99.1	101.6	102.5	102.4	102.3	101.8	102.2
	Hy-GP(2)	95.5	96.9	99.7	101.0	101.9	101.0	100.1	100.2
	Hy-GP(3)	94.8	96.9	100.9	102.3	102.2	100.3	101.3	102.3
	Hy-SH(3)	79.6	88.4	98.2	101.9	102.1	100.8	102.3	101.8
2,3,6-Trichlorophenol	PRP-1	83.8	93.0	99.8	100.1	101.4	100.5	99.7	101.0
	PLRP-S	84.2	94.5	99.9	100.4	101.5	100.2	101.3	100.8
	Hy-GP(2)	79.5	91.8	99.7	101.6	100.9	101.3	101.7	101.9
	Hy-GP(3)	88.8	95.2	99.2	100.6	100.7	99.8	99.8	100.7
	Hy-SH(3)	80.1	84.8	95.5	102.6	101.9	101.2	101.8	101.7
2,3,5-Trichlorophenol	PRP-1	88.2	97.2	100.4	99.8	100.2	101.1	100.8	101.2
	PLRP-S	86.4	96.7	101.0	100.5	101.2	100.7	101.4	100.6
	Hy-GP(2)	87.0	96.9	102.5	101.1	101.8	102.7	102.6	102.9
	Hy-GP(3)	85.0	95.9	101.3	99.8	99.7	100.4	102.4	101.8
	Hy-SH(3)	83.6	92.8	100.1	101.3	101.2	101.9	102.7	102.1
2,3,4-Trichloroaniline	PRP-1	90.7	99.5	100.6	99.6	100.2	99.8	99.4	100.7
	PLRP-S	88.2	99.4	99.8	100.5	99.6	100.4	101.4	100.6
	Hy-GP(2)	85.4	95.4	99.2	101.3	101.4	100.7	101.6	101.3
	Hy-GP(3)	82.1	90.4	99.8	99.4	99.5	99.8	101.2	101.2
	Hy-SH(3)	79.5	80.4	92.2	99.1	100.6	101.7	100.4	101.8
2,3,4,6-Tetrachlorophenol	PRP-1	84.4	97.2	100.8	99.6	100.4	101.3	100.7	101.2
	PLRP-S	83.2	96.7	101.3	99.8	100.5	100.8	101.4	100.6
	Hy-GP(2)	82.1	91.2	99.4	101.0	99.4	98.4	98.1	98.9
	Hy-GP(3)	81.6	90.7	100.5	102.4	101.5	102.6	101.0	102.1
	Hy-SH(3)	80.8	92.2	99.5	101.8	100.5	101.2	100.0	102.8
2,4,6-Trichloroaniline	PRP-1	79.6	91.8	99.8	100.5	99.6	101.4	102.5	101.8
	PLRP-S	80.2	92.4	99.6	99.8	100.2	99.6	101.1	100.6
	Hy-GP(2)	78.2	89.8	97.2	100.1	101.1	101.4	100.3	101.3
	Hy-GP(3)	75.3	84.0	91.9	99.3	99.8	98.4	100.6	100.1
	Hy-SH(3)	75.4	89.8	94.8	99.8	101.1	101.9	102.0	101.7
Pentachlorophenol	PRP-1	57.4	69.5	80.6	94.2	99.8	100.4	99.2	99.7
	PLRP-S	55.7	71.8	82.7	95.4	100.6	100.0	98.9	100.3
	Hy-GP(2)	55.9	69.2	80.9	93.8	98.2	99.5	100.6	100.5
	Hy-GP(3)	56.9	64.0	81.2	92.4	98.1	98.2	97.5	98.2
	Hy-SH(3)	47.9	61.1	82.2	94.4	98.1	99.5	99.6	98.9
2,3,5,6-Tetrachloroaniline	PRP-1	65.9	72.8	83.8	93.7	96.9	100.1	99.6	99.8
	PLRP-S	65.0	73.4	84.0	92.8	95.4	99.7	100.1	99.9
	Hy-GP(2)	67.2	75.6	82.2	93.4	96.8	98.2	98.9	99.5
	Hy-GP(3)	61.8	69.6	83.5	90.5	94.4	99.9	98.3	98.9
	Hy-SH(3)	60.2	67.3	84.0	93.9	96.3	99.1	99.4	100.1
2,6-Di-*tert*.-butyl-4-methylphenol	PRP-1	68.1	80.4	89.7	93.8	96.4	99.7	101.2	100.4
	PLRP-S	69.7	79.6	90.2	94.1	95.9	99.8	101.2	100.1
	Hy-GP(2)	64.8	76.8	87.4	96.2	99.7	100.4	99.0	100.6
	Hy-GP(3)	68.0	78.9	88.9	94.4	96.3	99.7	102.1	98.9
	Hy-SH(3)	62.4	72.6	79.2	92.8	96.1	99.5	99.4	98.2

[a] Analytes are ordered according to their retention time.
[b] The fortification level is 2 μg/l. All other parameters according to the text.

slightly broad peak (Fig. 2c). The breakthrough volumes of the polar analytes, however, were smaller compared to those of the 3 mm I.D. cartridges (Table 2 and Fig. 1), because of the less sorbent material contained in the cartridges. The back pressure during sample loading was also higher and the sample

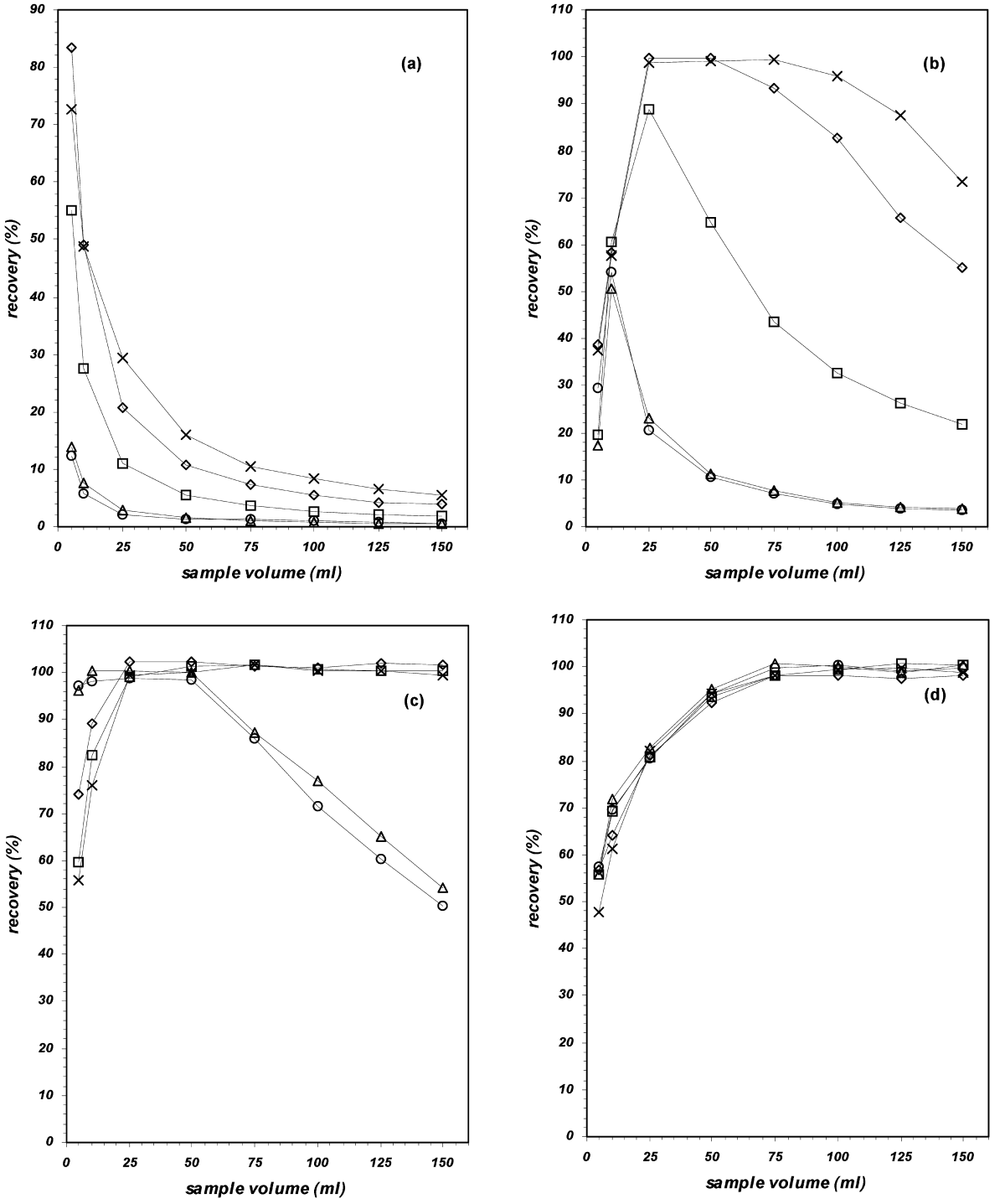

Fig. 1. Analyte % recovery vs. sample volume curves for aniline (a), phenol (b), 2-chlorophenol (c) and pentachlorophenol (d) for the PRP-1 10×3 mm (○), PLRP-S 10×2 mm (△), Hysphere-SH 10×3 mm (×), Hysphere-GP 10×3 mm (◇) and Hysphere-GP 10×2 mm (□) cartridges. The experimental conditions are given in the text.

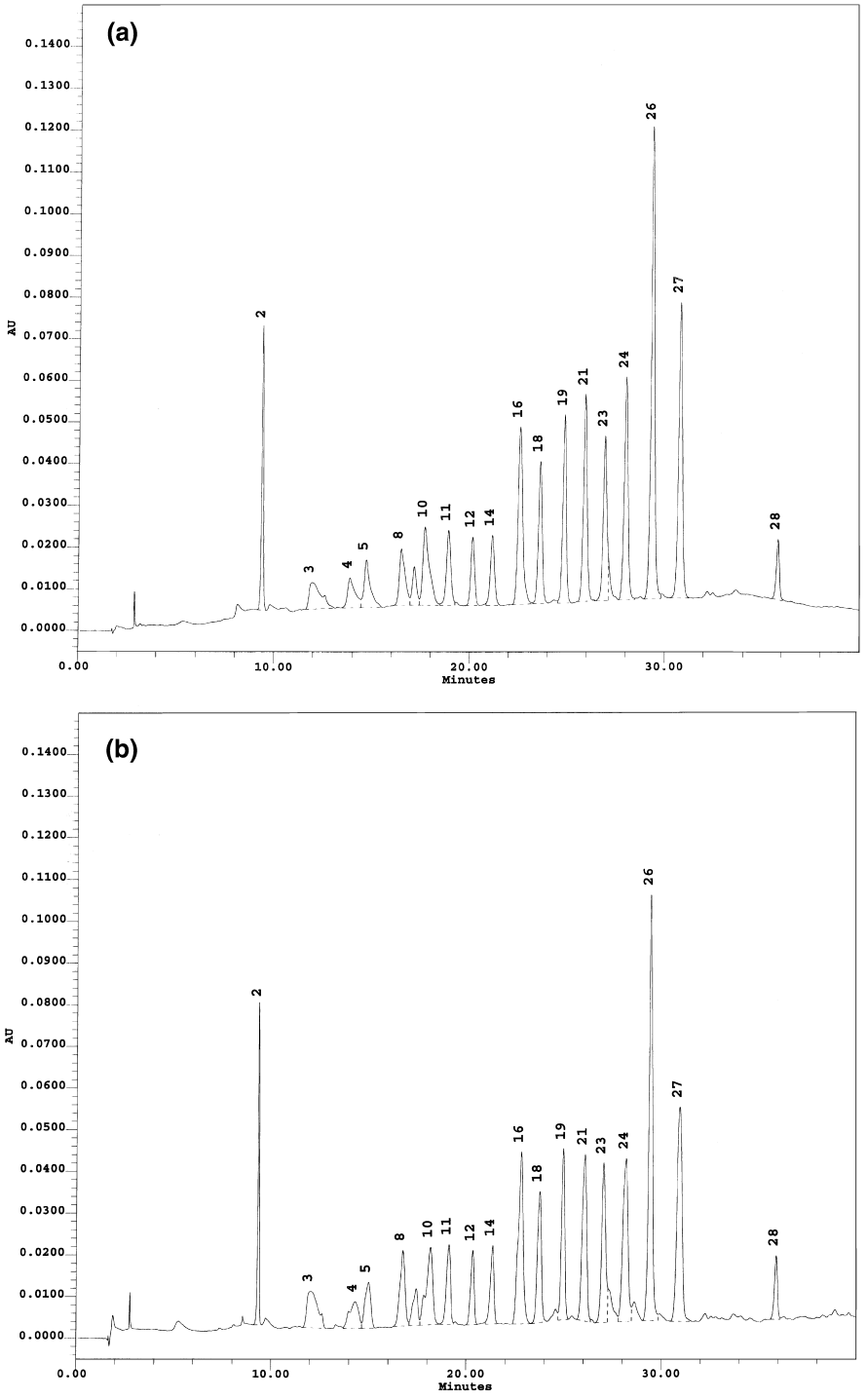

Fig. 2. Chromatograms of HPLC water sample spiked at the 2 μg/l level and processed on Hysphere-SH 10×3 mm (a), Hysphere-GP 10×3 mm (b) and Hysphere-GP 10×2 mm (c) cartridges; data from diode-array detection at 215 nm. Analyte peaks are designated as in Table 3. The experimental conditions are given in the text.

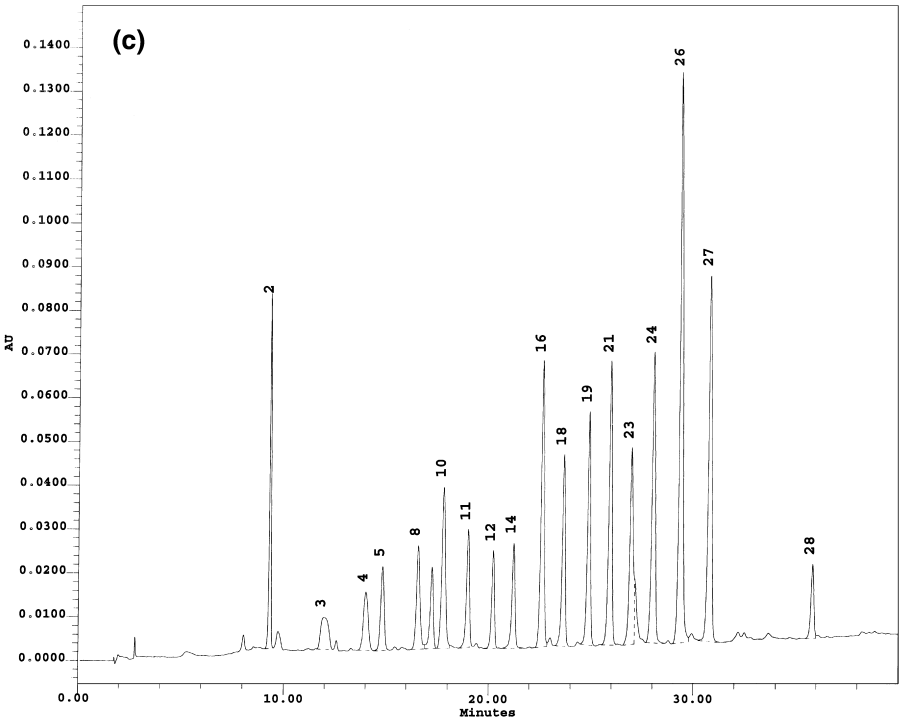

Fig. 2. (*continued*).

loading rate had not to exceed 2 ml/min in order for the analytical system to operate regularly. The Hysphere-GP material packed in 2 mm I.D. cartridges was used in all further studies.

An important aspect of the Hysphere-GP and Hysphere-SH cartridges is that the analyte peak shape is dependent on the sample volume especially for the polar and moderately polar analytes. For sample volumes up to 10 ml the peak shape was very good for all the compounds and comparable to that of the PRP-1 and PLRP-S cartridges. As the sample volume increased up to 150 ml the analyte peak shapes were gradually distorted.

As it can be seen from the data presented in Table 2 the recoveries for some analytes are low at low sample volumes and increase as the sample volume is increased. For the apolar analytes (Fig. 1d) this can be attributed to the adsorption onto the transfer lines, but for the polar analytes (Fig. 1b and c) no explanation seems to be suitable at present and further work must be devoted to this peculiarity.

A sample volume of 50 ml was selected in order to have reasonable overall analysis times. Higher sample volumes could be used however without any significant worsening in the detection limits of the polar compounds due to early breakthrough [27]. Using higher sample volumes, better detection limits could be achieved however for the non-polar analytes.

Sample pH was set at pH=3 in order to avoid the deprotonation of the slightly acidic phenols. Moreover the recovery of the studied anilines was evaluated at pH 3 and pH 8 and no significant differences were observed due to possible protonation of aniline molecules at pH 3.

3.2. Validation data

The proposed analytical method has been validated with Axios river water (Central Macedonia, Greece), that receives industrial, communal and agricultural wastes. A summary of the performance of the method is given in Table 3.

The recoveries for all the compounds are higher than 75% except for aniline (6%), phenol (50%) and 3-chlorophenol (67%).

Table 3
Mean analyte[a] recoveries (RSD, $n=3$) and identification limits in Axios river water[b]

	Analyte	t_R (min)	λ_{quan} (nm)	Recovery (%) (RSD, %, $n=3$)		Identification limit (μg/l)
				0.2 μg/l	2 μg/l	
1	Aniline[c]	5.63	–	nd[d]	6 (12)	0.5
2	Caffeine	9.46	280	104 (4)	101 (1)	0.05
3	Phenol	12.42	215	nd	50 (5)	1
4	4-Nitroaniline	14.52	250	98 (2)	98 (2)	0.2
5	4-Nitrophenol	15.33	280	111 (3)	99 (1)	0.05
6	4-Chloroaniline	15.97	250	102 (4)	97 (1)	0.1
7	2-Nitroaniline	16.70	280	101 (3)	97 (2)	0.1
8	2-Chlorophenol	16.89	215	89 (7)	77 (2)	0.1
9	3-Chlorophenol	17.94	215	71 (9)	67 (6)	0.1
10	2-Nitrophenol	17.99	280	110 (2)	103 (2)	0.05
11	2,4-Dimethylphenol	19.02	215	102 (2)	101 (3)	0.1
12	4-Chloro-3-methylphenol	20.14	215	113 (7)	101 (4)	0.1
13	2,3-Dichlorophenol	20.60	215	104 (5)	104 (1)	0.1
14	2,5-Dichlorophenol	21.13	215	99 (1)	97 (6)	0.1
15	3,4-Dichloroaniline	21.80	250	101 (3)	96 (2)	0.1
16	2,3-Dichloroaniline	22.53	250	119 (5)	105 (4)	0.1
17	2,3,4-Trichlorophenol	23.93	215	103 (4)	100 (1)	0.1
18	2,3,6-Trichlorophenol	23.59	215	102 (4)	105 (2)	0.1
19	2,3,5-Trichlorophenol	24.87	215	129 (2)	102 (1)	0.1
20	3,4,5-Trichloroaniline	25.83	250	102 (6)	98 (1)	0.05
21	2,3,4-Trichloroaniline	25.95	250	115 (5)	102 (1)	0.05
22	2,4,5-Trichloroaniline	26.91	250	103 (4)	101 (1)	0.05
23	2,3,4,6-Tetrachlorophenol	26.98	215	118 (5)	106 (3)	0.05
24	2,4,6-Trichloroaniline	28.01	250	106 (1)	101 (1)	0.05
25	2,3,4,5-Tetrachloroaniline	29.68	215	99 (7)	95 (2)	0.05
26	Pentachlorophenol	29.71	215	114 (3)	97 (1)	0.05
27	2,3,5,6-Tetrachloroaniline	30.85	215	102 (4)	87 (4)	0.05
28	2,6-Di-*tert*.-butyl-4-methylphenol	36.17	215	99 (3)	88 (2)	0.1

[a] Analytes are ordered according to their retention time.
[b] Data acquired by diode-array detection.
[c] Aniline is quantitated by electrochemical detection. The value in last column is the detection limit.
[d] ''nd'' means not detected.

For the diode-array detector the identification limit for a compound has been taken by convention as the lowest fortification level at which the compound is detected with a signal-to-noise ratio (S/N) >3 and its spectrum is identified with a predetermined match degree when compared with the library spectrum. For the electrochemical detector the detection limit has been taken as the lowest fortification level at which the compound is detected with $S/N>3$.

The identification limits for all the compounds range between 0.05 and 0.2 μg/l except for phenol that has an identification limit of 1 μg/l, because of its low recovery and its relatively broad peak. Aniline is detected by electrochemical detection only and has a detection limit of 0.5 μg/l because of its very low recovery.

A typical chromatogram of an Axios river water sample spiked at the level of 1 μg/l is shown in Fig. 3.

3.3. Stability studies

The stability of the compounds included in this study at the sorbed state onto the Hysphere-GP (10×2 mm) cartridges has been evaluated for a 3-month time period under three different temperature regimes [deep freeze (−24°C), refrigeration (~4°C) and 20°C]. Aliquots of 50 ml from an HPLC

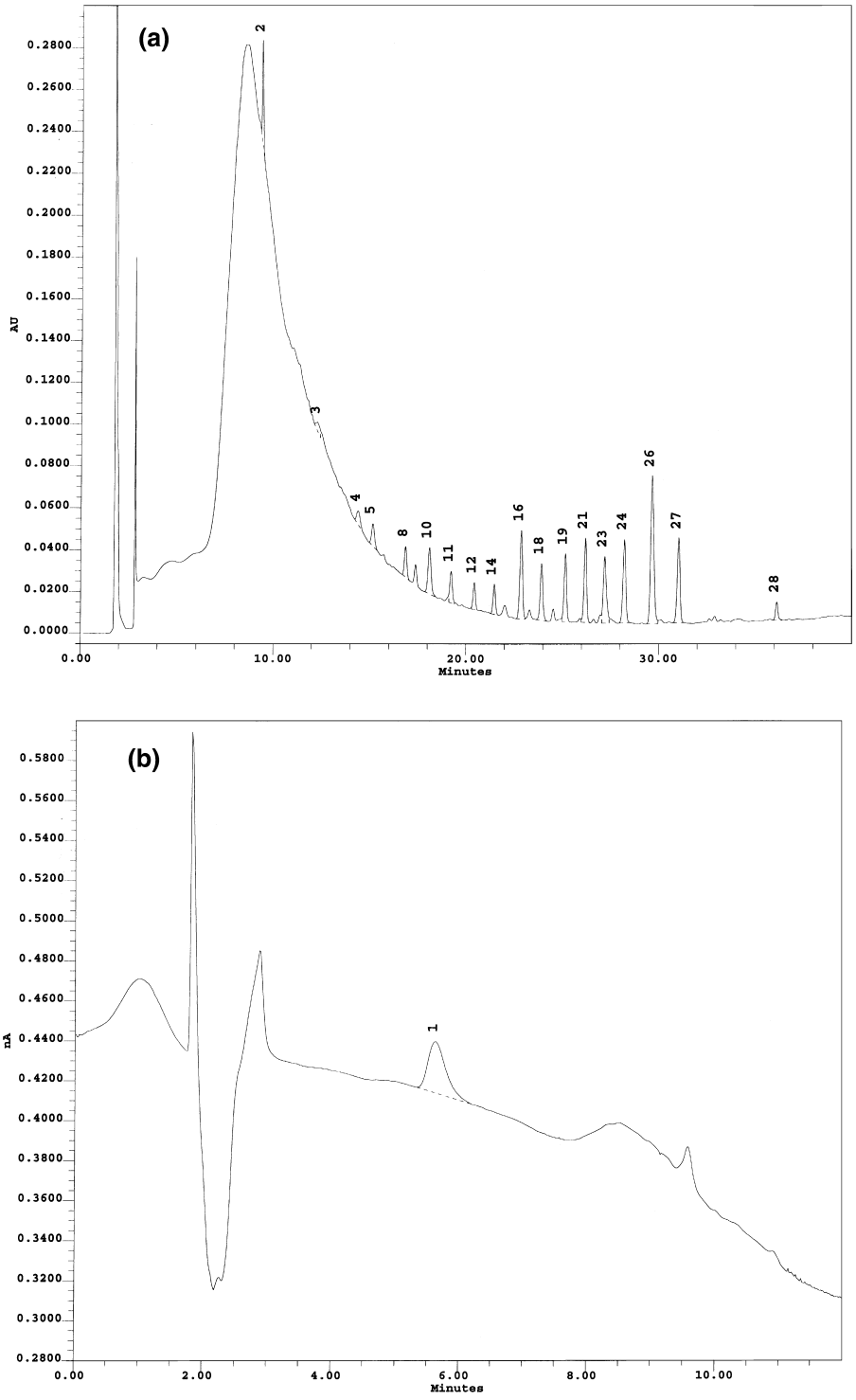

Fig. 3. Chromatograms of Axios river water spiked at the 1 μg/l level; data from diode-array detection at 215 nm (a) and electrochemical detection (b). Analyte peaks are designated as in Table 3. The experimental conditions are given in the text.

water sample spiked at the 10 μg/l level were loaded onto Hysphere-GP (10×2 mm) cartridges and the cartridges were closed in small plastic bags and immediately stored at the desired temperature. The relative amounts recovered for all the compounds under study at 1-, 2-, 4-, 8- and 12-week intervals are shown in Table 4. The acquired chromatograms of a HPLC water sample containing 19 compounds preserved under deep freeze and refrigerator conditions are shown in Fig. 4.

From the results shown in Table 4 phenols seem to be relatively stable at the absorbed state even at the 20°C temperature. Even phenol (one of the most volatile phenolic compounds) can be preserved for at least 3 months at 20°C without any significant losses. These results are better than those reported in the literature for the stability of phenolic compounds on the Isolute ENV sorbent [21], where more than 20% losses were observed after a 1-month period at room temperature for all the compounds under investigation.

Substituted anilines are less stable than the phenolic compounds. Plots for some selected anilines are shown in Fig. 5. Aniline that is the most volatile compound of this group is the most unstable analyte. It cannot be preserved at all even for a 1-week time period at 20°C.

From the data shown in Table 4 it derives that the stability of the various substituted anilines depends on the nature, the number and the position of the substituents. Nitroanilines are more stable than the chloroanilines (Fig. 5a and b). The stability of the substituted anilines increases with the number of the substituents. As it can be seen in Table 4 trichloroanilines are more stable than the dichloroanilines and these are more stable than the monochloroanilines. The position of a certain substituent also affects the stability of the substituted anilines. For example 3,4-dichloroaniline is less stable than the 2,3-dichloroaniline (Fig. 5c and d). Analyte losses are primarily due to analyte evaporation and depend on the vapor pressure of the analytes and on their affinity to the sorbent. The nature, the number and the position of the substituents affects the volatility and the other physicochemical properties of the analytes, which control their affinity to the sorbent. The affinity of the analytes to the sorbent is also depending on the physicochemical properties of the sorbent.

Stability studies were also carried out for target analytes present in field samples of industrial wastes (the samples donated by Dr. Barcelo from CSIC, Barcelona, Spain). The acquired data from the analysis of one of these samples just after delivery and after 1 month of storage at 20°C are presented in Table 5. The results shown in Table 5 confirm those of Table 4; the two detected phenolic compounds are very stable at the sorbed state at 20°C after 1 month of storage.

Except for the analyte stability two other important observations were made during the stability experiments regarding the preservation of water samples onto SPE cartridges. The first observation is the variation of the peak shape of some analytes with time and the second the appearance of matrix interference peaks on the chromatograms of the preserved samples.

The peak shape of some phenols (especially those of moderate polarity) is changing with the storage time. A small shoulder appears gradually at the end of the peaks and a small separate peak is formed after 3 months storage. This phenomenon depends on the storage temperature and occurs mainly at 20°C and to a less degree at ~4°C; at −24°C is almost negligible (Fig. 4). This implies some kind of surface diffusion or some other kind of mobility of the analytes into the SPE cartridge during storage.

A lot of matrix interference peaks appear also on the chromatograms of the preserved samples. The number and the height of the interference peaks are increased with increasing storage time and temperature. This phenomenon is more intense at 20°C, less intense at ~4°C and almost negligible at −24°C (Fig. 4).

If the Hysphere-GP (10×2 mm) cartridges are going to be used for sample storage, the desired storage conditions should be those of deep freeze.

3.4. Application data

The proposed analytical method has been applied for the analysis of surface and ground water samples coming mainly of Northern Greece. Caffeine and 2,6-di-*tert*.-butyl-4-methylphenol were the most frequently detected compounds. Their presence in various water samples has also been validated with other analytical techniques in our laboratory (GC–ion trap MS) and this was the main reason for these com-

Table 4
Analyte[a] stability at the sorbed state onto the Hysphere-GP (10×2 mm) cartridges under deep freeze (−24°C), refrigeration (~4°C) and 20°C for a 3-month time period.

Analyte	Temperature (°C)	Mean relative recovery (%, $n=3$)				
		1 week	2 weeks	1 month	2 months	3 months
Aniline	−24	67.5	58.1	49.1	25.1	14.8
	~4	12.7	nd[b]	nd	nd	nd
	20	nd	nd	nd	nd	nd
Caffeine	−24	99.0	98.7	99.1	99.4	100.4
	~4	99.2	98.9	98.6	99.5	100.2
	20	98.1	98.3	100.2	100.8	99.7
Phenol	−24	100.7	99.5	100.1	98.7	99.0
	~4	99.3	99.8	100.0	100.2	99.8
	20	100.5	102.1	101.0	100.2	100.7
4-Nitroaniline	−24	99.2	98.9	98.6	99.2	98.5
	~4	93.0	81.4	70.8	61.8	53.9
	20	77.1	67.3	59.3	46.6	34.5
4-Nitrophenol	−24	99.1	98.6	99.2	98.0	99.4
	~4	98.4	97.8	97.3	96.7	97.7
	20	100.0	100.8	98.9	98.3	99.3
4-Chloroaniline	−24	82.9	75.4	70.1	62.4	59.3
	~4	62.2	50.0	39.2	21.0	14.2
	20	46.6	28.1	13.5	nd	nd
2-Nitroaniline	−24	98.1	98.0	100.5	97.8	100.3
	~4	100.2	98.4	99.7	101.3	100.1
	20	99.3	100.3	98.6	97.7	98.0
2-Chlorophenol	−24	100.8	99.7	100.1	99.5	99.9
	~4	94.0	94.3	94.8	93.7	95.8
	20	94.5	91.4	90.9	92.2	94.3
3-Chlorophenol	−24	99.3	100.2	101.1	99.0	100.2
	~4	100.5	100.2	98.3	101.8	99.3
	20	101.6	101.5	100.6	99.6	101.9
2-Nitrophenol	−24	100.2	99.2	99.8	98.0	99.6
	~4	98.6	99.3	98.2	97.1	99.7
	20	94.1	94.8	94.1	92.2	93.5
2,4-Dimethylphenol	−24	100.3	99.7	101.2	100.6	99.8
	~4	98.4	98.0	97.8	97.0	100.5
	20	97.7	97.3	97.1	98.0	97.6
4-Chloro-3-methylphenol	−24	97.2	98.2	101.0	98.6	99.9
	~4	98.4	98.4	98.5	97.5	98.7
	20	100.3	100.1	98.0	99.1	100.0
2,3-Dichlorophenol	−24	100.1	99.7	98.1	99.2	101.2
	~4	100.0	97.8	97.8	97.3	96.5
	20	97.8	98.8	95.8	94.1	94.2
2,5-Dichlorophenol	−24	100.5	99.6	101.9	98.7	98.9
	~4	99.0	98.5	97.6	98.4	97.1
	20	100.7	100.1	98.5	99.5	100.3

Table 4. Continued

Analyte	Temperature (°C)	Mean relative recovery (%, $n=3$)				
		1 week	2 weeks	1 month	2 months	3 months
3,4-Dichloroaniline	−24	98.0	96.5	94.3	90.0	85.9
	~4	89.4	73.0	61.9	50.4	39.3
	20	71.4	59.0	44.5	32.1	22.4
2,3-Dichloroaniline	−24	98.4	97.9	98.3	98.1	98.9
	~4	92.6	87.4	83.1	80.0	77.2
	20	90.5	83.3	79.8	74.5	70.3
2,3,4-Trichlorophenol	−24	100.7	98.2	100.0	99.7	100.9
	~4	98.9	98.4	98.5	99.5	100.0
	20	96.0	97.1	98.1	95.2	94.5
2,3,6-Trichlorophenol	−24	97.9	98.5	99.2	98.0	99.2
	~4	97.7	98.4	98.3	99.4	100.6
	20	98.4	98.7	99.5	97.5	98.5
2,3,5-Trichlorophenol	−24	99.7	99.1	98.7	99.4	100.6
	~4	98.8	98.9	99.5	99.4	100.2
	20	99.7	99.7	100.8	98.0	99.2
3,4,5-Trichloroaniline	−24	98.7	98.6	96.5	95.2	95.1
	~4	95.7	94.3	93.5	92.0	90.3
	20	93.2	90.0	86.1	80.5	75.3
2,3,4-Trichloroaniline	−24	98.4	98.6	99.5	98.8	100.0
	~4	98.9	98.5	99.1	100.7	99.7
	20	99.8	100.2	98.1	99.6	98.2
2,4,5-Trichloroaniline	−24	98.6	98.0	97.8	98.5	98.0
	~4	96.6	93.9	92.6	91.4	90.6
	20	94.9	89.3	87.0	83.1	80.5
2,3,4,6-Tetrachlorophenol	−24	99.6	99.0	98.5	98.9	99.4
	~4	100.8	99.8	99.2	100.5	99.3
2,4,6-Trichloroaniline	20	98.7	101.9	98.8	100.7	100.3
	−24	101.7	99.8	97.6	99.7	100.7
	~4	101.1	99.7	99.1	99.4	100.0
	20	98.5	98.3	96.8	97.9	98.8
2,3,4,5-Tetrachloroaniline	−24	96.5	97.0	96.9	97.9	98.2
	~4	95.8	95.7	94.5	94.6	95.9
	20	95.8	98.9	100.7	98.6	97.3
Pentachlorophenol	−24	97.4	98.2	98.3	99.1	100.0
	~4	99.6	98.7	98.2	99.5	100.2
	20	98.1	97.5	100.5	97.6	98.2
2,3,5,6-Tetrachloroaniline	−24	100.8	100.1	99.0	98.6	100.2
	~4	100.7	99.4	99.9	98.0	99.4
	20	98.3	98.0	99.9	99.9	99.2
2,6-Di-*tert*.-butyl-4-methylphenol	−24	98.3	98.6	98.0	97.9	99.2
	~4	97.5	97.9	98.8	95.1	97.8
	20	95.5	93.9	96.3	95.2	96.5

[a] Analytes are ordered according to their retention time.
[b] ''nd'' means not detected.

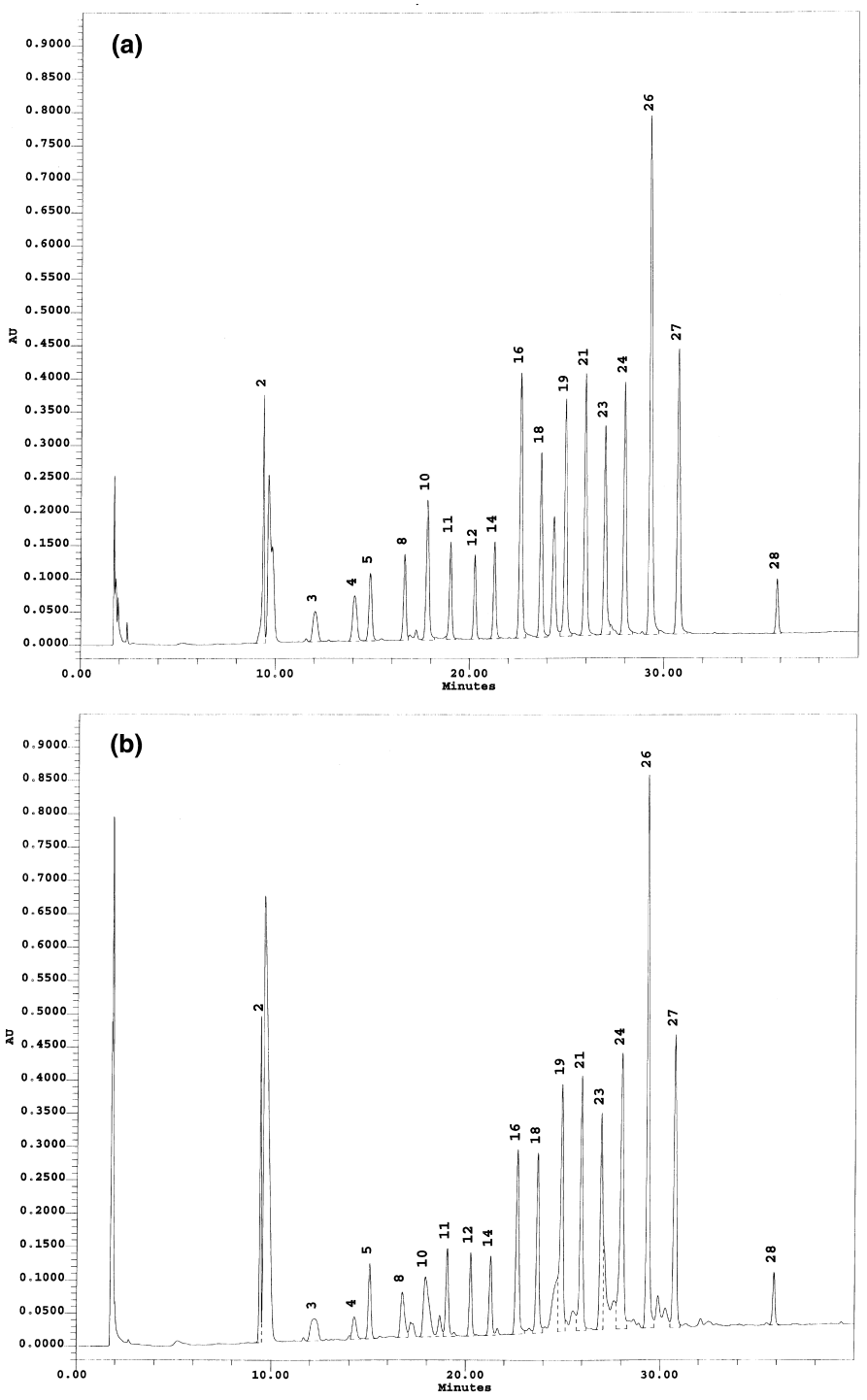

Fig. 4. Chromatograms of HPLC water sample spiked at the 10 μg/l level and preserved on Hysphere-GP 10×2 mm cartridges for 3 months under deep freeze (a) and refrigerator (b) conditions; data from diode-array detection at 215 nm. Analyte peaks are designated as in Table 3. The experimental conditions are given in the text.

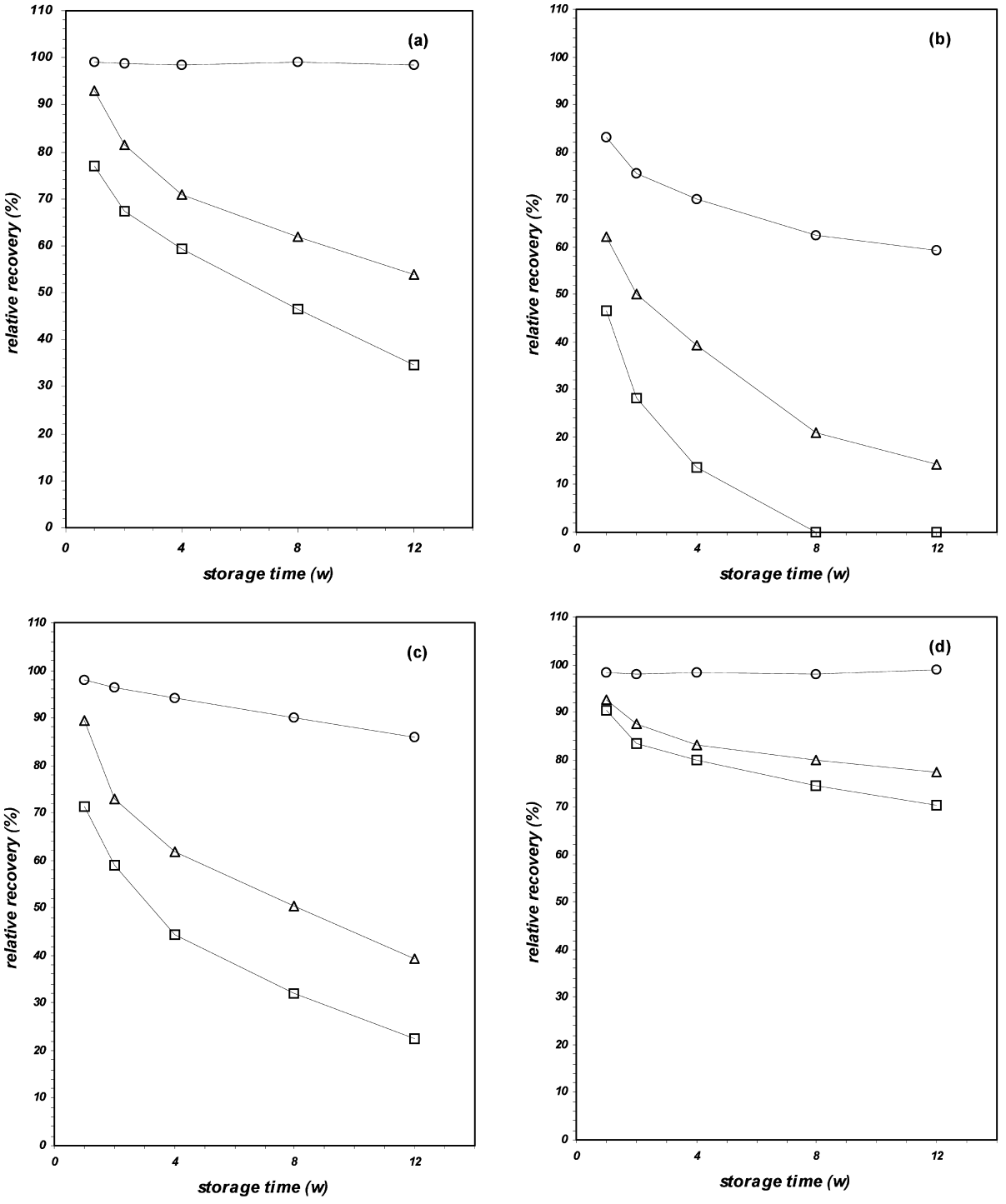

Fig. 5. Relative % recovery vs. storage time curves for 4-nitroaniline (a), 4-chloroaniline (b), 3,4-dichloroaniline (c) and 2,3-dichloroaniline (d) under deep freeze (○), refrigerator (△) and 20°C (□) conditions. The experimental conditions are given in the text.

Table 5
Results from stability studies for target analytes present in a waste water sample[a]

Analyte	Concentration (μg/l) (RSD, %)		Relative recovery (%)
	Initial	1 month, 20°C	
Phenol	2277 (0.2)	2291 (2.4)	100.6
4-Chloro-4-methylphenol	836 (0.3)	822 (1.0)	98.3

[a] The sample has been 20-fold diluted and only 10 ml of the sample was processed.

pounds to be included in the target analyte list of this study. A chromatogram of Axios river water (sample taken on 12 May 2000), where caffeine at 0.16 μg/l and 2,6-di-*tert*.-butyl-4-methylphenol at 0.32 μg/l level were found is shown in Fig. 6. The repeatability of the proposed method when analyzing real samples was very good with the corresponding RSD values being less than 10% ($n=3$).

4. Conclusions

A multiresidue method has been developed for the simultaneous analysis of substituted anilines and phenols in water matrices based on automated on-line SPE followed by HPLC determination with tandem diode-array and electrochemical detection.

The Hysphere-GP and the Hysphere-SH materials, which were evaluated to be used for the SPE of anilines and phenols from water matrices, showed very good recoveries for the tested compounds, but considerable band broadening occurred when were combined on-line with liquid chromatographic analysis. Reducing cartridge diameter proved a good way to improve the chromatographic performance, due to better sample refocusing onto the analytical column.

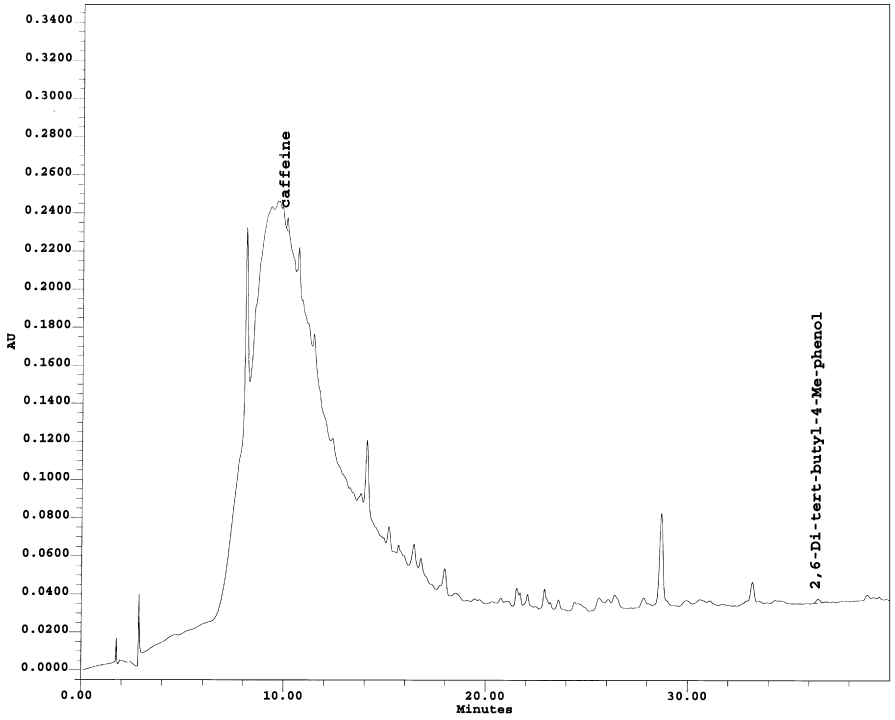

Fig. 6. Chromatogram of Axios river water (sample taken on 12 May 2000) analyzed by the proposed method; data from diode-array detection at 215 nm. The experimental conditions are given in the text.

The SPE cartridges that were found to give better results in terms of method sensitivity and selectivity were Hysphere-GP (10×2 mm) and were therefore selected to be used in the proposed method.

The identification limits of the analytical method are those of the diode-array detector and range from the 0.05 to 0.2 μg/l range for all the tested compounds except for phenol that has an identification limit of 1 μg/l. The electrochemical detector provides only additional evidence for the identity of the detected analytes. Even though it was proved very selective for anilines and phenols, still a lot of matrix peaks interfering with the analyte peaks appeared on the real sample chromatograms. Only aniline is detected and quantitated by the electrochemical detector (because its UV absorbance is very low) and has a detection limit of 0.5 μg/l.

The repeatability of the method was very good judging from the RSD values of the compound recovery, which did not exceed 10% (n=3) even at the 0.1 μg/l concentration.

Finally, the stability of the tested compounds at the sorbed state onto the Hysphere-GP (10×2 mm) cartridges was evaluated. Phenols were found to be very stable even for a 3-month storage period at 20°C. Anilines were not so stable while aniline and 4-chloroaniline were the least stable compounds. If these cartridges are going to be used for sample stabilization and storage, the proposed storage conditions are those of deep freeze.

Acknowledgements

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