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Journal of Chromatography A, 755 (1996) 67–74

JOURNAL OF CHROMATOGRAPHY A

On-line solid-phase extraction coupled to supercritical fluid chromatography to determine phenol and nitrophenols in water

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Received 19 December 1995; revised 18 June 1996; accepted 18 June 1996

Abstract

Determining of phenol and nitrophenols using solid-phase extraction on-line coupled to supercritical fluid chromatography (SFC) is studied. SFC quickly separated the compounds studied, in less than 6 min, and solid-phase extraction was used to decrease the limits of detection. C_{18}, PLRP-S and a highly cross-linked styrene–divinylbenzene copolymer in a 10×3 mm I.D. laboratory-packed precolumn were tested comparatively as sorbents in the preconcentration step. Tetrabutylammonium bromide was used as ion-pair reagent in the extraction process to increase breakthrough volumes, mainly for phenol. Performance of the method was checked with tap and river waters.

Keywords: Water analysis; Environmental analysis; Ion-pairing reagents; Phenols; Nitrophenols

1. Introduction

Phenol and nitrophenols are of great environmental interest and have been found in water as common pollutants. They are currently formed through different processes: industrially, during the manufacture of dyes or explosives; biogeochemically through the degradation of natural organic compounds, and mainly through the degradation of pesticides [1–3].

Determination of phenol and nitrophenols in water has become increasingly important in the last few years because of growing knowledge about their toxicity, even at low concentrations, especially for aquatic organisms.

The analyses were performed using HPLC or GC techniques after some preconcentration procedures based on liquid–liquid extraction. Methods based on GC are time consuming because they usually require a derivatization step to enhance the volatility of the phenolic compounds [4–7]. Although in a recent paper Wennrich et al. [8] used a highly deactivated separation system for the quantitative GC analysis of underivatized nitrophenols, the analysis time was still high, almost 40 min. HPLC methods have been the most widely used, due to the fact that the derivatization step is not necessary, but even using amperometric detectors they are not sensitive enough to detect these compounds at $\mu g\ l^{-1}$ level, so there must be a preconcentration process [9,10]. Of the different preconcentration techniques, nowadays solid-phase extraction (SPE) is the most used. This technique has been on-line coupled to GC [11] and to HPLC [12–14], the coupling in the latter case being less complex.

In previous works, we have been able to determine phenol and nitrophenols at $\mu g\ l^{-1}$ levels using these

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0021-9673/96/$15.00
PII S0021-9673(96)00574-2

techniques [10,15], but when real water samples were analysed a band peak appeared at the beginning of the chromatogram which made it difficult to correctly quantify the phenol. A biosensor based method using on-line SPE has been developed recently by Burestedt et al. [16], and although phenol was determined at 1 $\mu g\ l^{-1}$ level only three phenolic compounds could be screened, and there were problems with the stability of the biosensor when organic solvents were used, so collecting phenolic compounds with different polarities in a single fraction was difficult.

In recent years, interest in the use of supercritical fluid chromatography (SFC) as a separation technique has been increasing rapidly because of the unique properties of supercritical fluids: their higher diffusivity and lower viscosity enable analysis to be 3 to 10 times faster than HPLC and they are very efficient at separation. On the other hand, they have relatively similar densities to liquids and viscosities comparable to gases, so SFC can be used to analyse a wide range of compounds, particularly those that are thermally labile, non volatile and of high molecular mass that cannot be satisfactorilly analysed by GC [17–19]. The most widely used supercritical fluid is CO_2, although for analyses of polar and high-molecular-mass solutes, polar modifiers such as methanol must be incorporated to increase the solvent strength [20].

So far, a few papers [20–24] have described the separation of phenolic compounds by studying the different parameters that affect the separation such as temperature, pressure or polar modifiers; but none of them have used on-line SPE to improve the detection limits. Taking into account that the desorption of the compounds by CO_2 can be more selective than in on-line SPE-HPLC, some interference from the matrix can be avoided, and the analysis time can also be reduced significantly using SFC.

The aim of this paper is to study the capability of SPE on-line coupled to SFC with diode array detection, in the rapid and sensitive determination of phenol and nitrophenols in water samples. Several columns and the influence of chromatographic conditions such as temperature, pressure, flow-rate and adding methanol in the mobile phase, were studied in order to separate the compounds. To decrease the detection limits of the method, SPE on-line coupled to SFC was tested. An ion-pair reagent was added to the samples to increase the breakthrough volumes of the compounds studied. Finally the performance of the method was checked with tap and river water samples.

2. Experimental

2.1. Equipment

The experiments were performed on a Hewlett-Packard (Palo Alto, CA, USA) Model G1205A supercritical fluid chromatograph equipped with an HP 7673 automatic injector with a 5-μl loop and an HP 1050 diode array detector. Chromatographic data were collected using an HP-SFC 3365 ChemStation, which was controlled by Windows 3.1 (Microsoft). The columns tested for carrying out chromatographic separation were 150×4.6 mm I.D. 5 μm HP Spherisorb ODS-2, 250×4.6 mm I.D. 5 μm HP LiChrospher Diol, and HP Hypersil Silica.

2.2. Reagents and standards

The phenolic compounds studied are: phenol (Ph), 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP) and 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP). All phenolics studied were supplied by Aldrich Chemie (Beerse, Belgium). Standard solutions (2000 $mg\ l^{-1}$) of each compound were prepared in methanol–water (50:50) and stored in the refrigerator. Working solutions were prepared weekly by diluting these solutions with Milli-Q-quality water (Millipore, Bedford, MA, USA).

Carbon dioxide of 99.999 quality purchased from Carburos Metálicos (Madrid, Spain) was used as mobile phase and HPLC-grade methanol (Scharlau, Barcelona, Spain) as modifier. TBA (tetrabutylammonium bromide) from Aldrich (Beerse, Belgium) was used as ion-pair reagent in the extraction process. Helium 99.999 quality purchased from Carburos Metálicos was used to dry the sorbent in the precolumn before eluting the analytes.

NaOH was purchased from Probus (Badalona, Spain) to adjust the pH of sample before solid-phase extraction and HPLC quality methanol was used in the same process to clean the tubes and precolumn.

2.3. Chromatographic conditions

The phenolic compounds studied were separated using carbon dioxide as the mobile phase and methanol as modifier. The mobile phase was changed from 3% of methanol to 7% in 2 min, from 7% to 47% in 6 min and was returned to initial conditions in 1 min. So, the total analysis time was 9 min. The flow-rate was 2 ml min^{-1} and the outlet pressure was maintained constant during the analysis at 150 bar. The oven temperature was set at 40°C. For single-wavelength monitoring the detection was set at the optimum wavelength for each compound studied: 280 nm for 2-NP and Ph, 254 nm for 2-M-4,6-DNP and 2,4-DNP and 302 nm for 4-NP. The spectra were recorded between 210 and 350 nm.

2.4. On-line trace enrichment

The on-line trace-enrichment experiments were performed using two six-port rotary valves (Rheodyne, Cotati, CA, USA) connected in series to make the different steps of the preconcentration process possible: conditioning and activation of the precolumn, retention of the analytes, drying of the precolumn and elution of the compounds. To carry out the solid-phase extraction, three sorbents were tested by laboratory-packing them in a 10×3 mm I.D. precolumn purchased from the Free University (Amsterdam, Netherlands). The sorbents tested were 10 μm Spherisorb ODS-2 purchased from Teknokroma (Barcelona, Spain), 20 μm PLRP-S (Polymer Labs., Shropshire, UK) and 80–160 μm highly cross-linked styrene–divinylbenzene copolymer ENVI Chrom P (Supelco, Bellefonte, PA, USA). An Eldex Waters (Milford, MA, USA) pump was used to deliver the sample and the conditioning solutions.

The scheme of the equipment used is shown in Fig. 1 and the sequence followed in the extraction process is described in Table 1. The flow-rate used throughout the extraction process was 2 ml min^{-1}. Firstly, the preconcentration system was washed with 10 ml methanol to remove all solvents from the tubes. Then, the precolumn was cleaned and conditioned with 10 ml methanol. Next, tubes were washed with 10 ml of water–TBA and the precolumn was activated with 10 ml of the same solution. In the next step the sample was preconcentrated after washing the tubes and at the same time the tubes were dried with helium. Then, the precolumn was dried with 5 bar helium for the optimized time for the type of sorbent. The analytes trapped in the precolumn were desorbed in the backflush mode and on-line transferred to the analytical column. The precolumn was kept in-line the whole time.

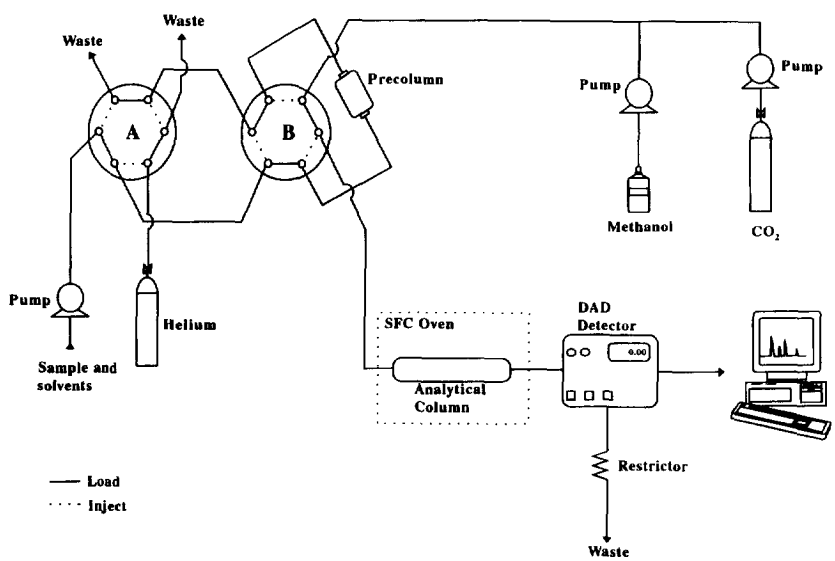

Fig. 1. Scheme of the equipment used.

Table 1
Sequence followed in the extraction process

Step	Event	Valve A	Valve B
1	Washing tubes with 10 ml methanol	Inject	Load
2	Conditioning precolumn with 10 ml methanol	Load	Load
3	Washing tubes with 10 ml water–TBA	Inject	Load
4	Activation precolumn with 10ml water–TBA	Load	Load
5	Washing tubes with 10 ml sample	Inject	Load
6	Sample preconcentration and driying tubes with helium	Load	Load
7	Drying precolumn with 5 bar helium	Inject	Load
8	Analyte desorption	Load	Inject

When river or tap water was analyzed, water was filtered through a 0.45-μm filter (MSI, Westboro, USA) before analysis. Also, 300 μl of a 10% solution of Na_2SO_3 was added for each 100 ml of tap water before analysis in order to eliminate free chlorine, which could react with phenol added as standard addition and produce chlorophenols [14].

In all cases, the pH of the samples was adjusted to 9.0 with 0.1 *M* NaOH and TBA was added at a concentration of 5 m*M* before the preconcentration step. These conditions were optimized in a previous paper.

3. Results and discussion

To carry out the separation of phenol and nitrophenols, different analytical columns were tested: C_{18}, silica and diol. C_{18} and silica columns had not been used before in the separation of these kinds of compound by SFC. These columns were evaluated by varying experimental conditions such as temperature, pressure, percentage of methanol and flow-rate in order to obtain the best chromatographic separation. The results were not good. Peak shapes were distorted and some compounds were not resolved even when nine silica columns or three C_{18} columns were connected in series.

The diol column was found to provide better separations. Different temperatures as well as pressures were tested but they had no influence on the selectivity and only the retention time changed slightly. The use of an additive (acetic or trifluoroacetic acids) in the mobile phase was studied in order to improve peak shapes [20]. The concentration was varied from 0.025% to 1%, but better peak shapes were not obtained, so the next studies were carried out without additive. The flow-rate was also varied between 1 and 4 ml min^{-1}. Some problems in the nozzle appeared when the flow-rate was higher than 3 ml min^{-1} and there were good separations in a short analysis time (6 min) using a flow-rate of 2 ml min^{-1} and the following methanol gradient: initial conditions 3%, at 2 min, 7% and at 6 min, 47%. The chromatogram corresponding to 50 mg l^{-1} for each compound studied under optimum conditions is shown in Fig. 2. The use of a diode array detector enabled us to detect each phenolic compound at maximum absorbance wavelength and also to compare the spectra of peaks in real samples with those of standards.

The range of linearity of response in the chromatographic method was between 1 and 150 mg l^{-1} for all compounds studied except Ph and 2,4-DNP whose linearity was between 5 and 150 mg l^{-1}. Regression coefficients were higher than 0.997 in all cases and the detection limits obtained considering $S/N=3$ were between 0.2 mg l^{-1} for 4-NP and 1 mg l^{-1} for Ph and 2,4-DNP.

In order to lower the detection limits, on-line solid-phase extraction was studied and various sorbents such as C_{18}, PLRP-S and ENVI-Chrom P were tested. The on-line coupling of these sorbents to HPLC for the analysis of phenolic compounds has already been studied by our group [25,26]. On-line systems have a higher automation potential, higher sensitivity and a lower organic solvent consumption. TBA was used as ion-pair reagent to increase

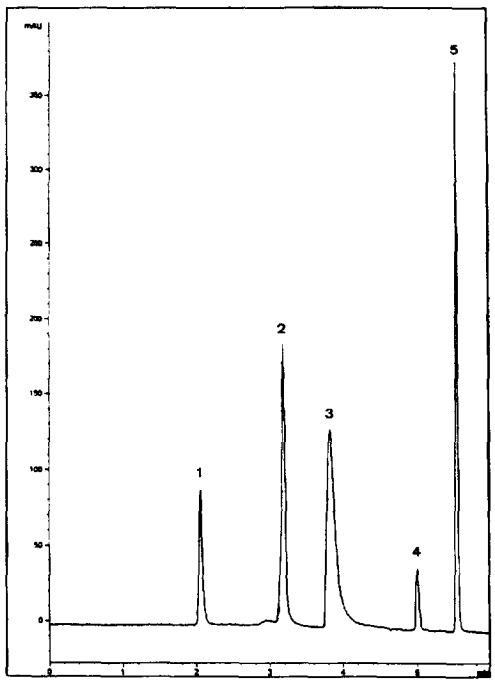

Fig. 2. Chromatogram for 50 mg l^{-1} of each compound studied under the optimum conditions found. (1) 2-NP; (2) 2-M-4,6-DNP; (3) 2,4-DNP; (4) Ph; (5) 4-NP.

breakthrough volumes, mainly for Ph, as has been described in previous papers [15,25]. Moreover, the ion-pair formed is less polar than the analyte so its solubility in CO_2 may be higher and easier the elution, which involves a decrease of broadening of the peaks.

Thus, drying time was optimized for each type of sorbent using helium at a pressure of 5 bar. The initial volume of preconcentrated sample was only 2 ml of a standard solution of 0.1 mg l^{-1} to prevent losses of compounds studied due to their breakthrough volumes, mainly when C_{18} is used. It was observed that, using the preconcentration step, the retention times of some compounds were slightly higher than the direct injection ones. However, chromatographic time did not increase by more than 6 min. This was mainly observed with the most acidic compounds studied, 2-M-4,6-DNP and 2,4-DNP (pK_a 4.35 and 4.09, respectively). This could be explained by the fact that the ion-pair formed was not broken by the mobile phase used in the SFC system and, instead, the phenolic compounds were separated as ion pairs. This effect was not observed when SPE was coupled to RPLC because the ion pair was destroyed by the acid present in the mobile phase. The rest of the compounds studied are less acidic and the ion pair is more easily broken. For all the sorbents tested, peaks broadened and resolution decreased when the drying time was lower than the optimum. On the other hand, times which were higher than the optimum led to some peaks being distorted, in particular the last one eluted. Using C_{18} or ENVI-Chrom P a double peak for the second compound corresponding to 2-M-4,6-DNP was observed. The optimum times found were 5 min for C_{18} and Envi-chrom P and 10 min for PLRP-S. As an example, chromatograms for different drying times using PLRP-S sorbent are shown in Fig. 3.

In order to check the possible matrix effect, tap and river water were preconcentrated in each precolumn. 2 ml of each real sample spiked with a standard addition of 0.1 mg l^{-1} of phenolic compounds were preconcentrated and the precolumn was

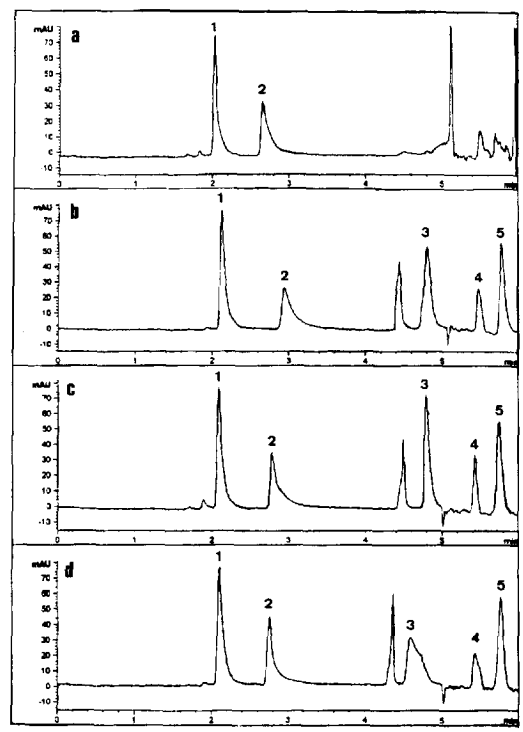

Fig. 3. Chromatograms for different drying times using PLRP-S sorbent. (a) 0 min; (b) 5 min; (c) 10 min and (d) 15 min. For more conditions see Section 2.3. For peak designation, see Fig. 2.

dried for the optimum time found. Slight band broadening of the peaks was observed in all cases compared to direct injection using Milli-Q quality water, but there were no losses in resolution. Using C_{18} or ENVI-Chrom P there was a higher distortion of the peak corresponding to 2-M-4,6-DNP so this compound could not be reliably quantified. This effect was not observed when PLRP-S was used, so it was selected for the next studies. It should be pointed out that with supercritical fluid chromatography no band peak appeared at the beginning of the chromatogram as happened when real samples were analysed with RPLC. This means that SFC correctly quantifies phenol while RPLC presents some limitations.

The breakthrough volumes of the compounds studied on the 10×3 mm PLRP-S precolumn were calculated by preconcentrating under optimum conditions different sample volumes prepared with Milli-Q quality water and the corresponding addition of TBA with the pH value adjusted to 9.0. From the results obtained, which are shown in Table 2, the volume selected to be preconcentrated was 20 ml because with higher volumes there were phenol losses. The recoveries obtained by preconcentrating 20 ml were higher than 90% for all compounds studied. Higher volumes were not tested because Ph recoveries decreased.

The analytical performance of the method was tested with tap and river water under the optimum conditions found.

The linearity of the method checked with tap water was between 1–40 $\mu g\ l^{-1}$ for 2-NP and 4-NP, 5–40 $\mu g\ l^{-1}$ for 2,4-DNP, 10–40 $\mu g\ l^{-1}$ for Ph and 1–20 $\mu g\ l^{-1}$ for 2-M-4,6-DNP. The regression coefficients (R^2) found were higher than 0.990. The repeatability and reproducibility between days of the

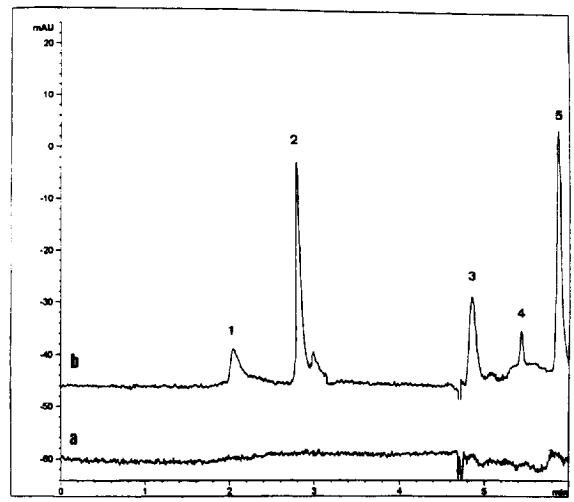

Fig. 4. Chromatograms obtained by preconcentrating 20 ml of tap water on a PLRP-S precolumn. (a) Without standard addition; (b) with a standard addition of 10 $\mu g\ l^{-1}$ for Ph and 5 $\mu g\ l^{-1}$ for the rest of the compounds studied. For more conditions see Section 2.3. For peak designation see Fig. 2.

method were checked with 20 ml of tap water spiked at a level of 10 $\mu g\ l^{-1}$ of each compound and the R.S.D.s found were between 1.1–8.8% and between 0.7–9.4% ($n=4$), respectively. The limits of detection, corresponding to a signal-noise ratio of about 3, were between 0.2 $\mu g\ l^{-1}$ for 4-NP and 1 $\mu g\ l^{-1}$ for Ph and 2,4-DNP. The precolumn was re-used several times as no cross-contamination was observed. Chromatograms for 20 ml tap water and 20 ml tap water spiked with 10 $\mu g\ l^{-1}$ for Ph and 5 $\mu g\ l^{-1}$ for the rest of compounds studied are shown in Fig. 4. It can be seen that no peak appeared at the same retention time as the compounds studied.

The same study was carried out with Pisuerga river water. The linearity of the method was between

Table 2
Mean recoveries ($n=3$), of on-line SPE with a 10×3 mm I.D. PLRP-S precolumn for different volumes of a solution of phenolic compounds in Milli-Q water with 5 m*M* TBA and a pH value of 9.0

Compounds	20 ml		25 ml		30 ml	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
2-NP	95	3.2	94	3.8	94	3.9
2-M-4,5-DNP	98	1.4	98	1.1	98	1.3
2,4-DNP	100	2.5	99	2.8	102	2.8
Ph	92	2.0	75	1.3	69	3.5
4-NP	100	2.2	101	3.2	100	2.4

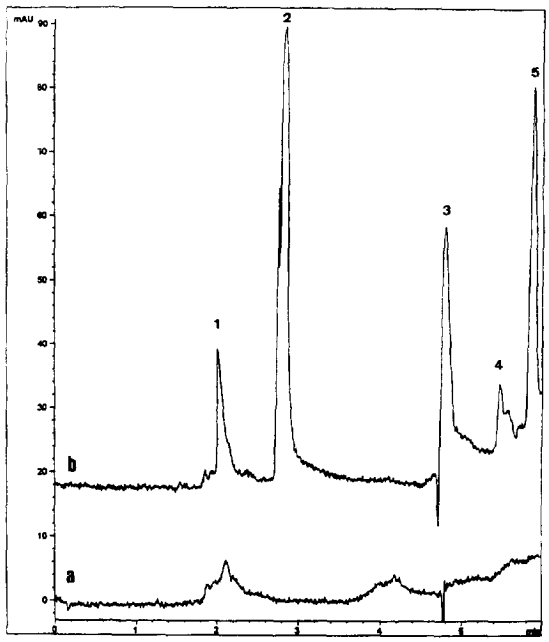

Fig. 5. Chromatograms obtained by preconcentrating 20 ml of Pisuerga river water on a PLRP-S precolumn. (a), with no standard addition; (b), with a standard addition of 5 $\mu g\ l^{-1}$ for 2-NP and 4-NP and 10 $\mu g\ l^{-1}$ for the rest of the compounds studied. For more conditions see Section 2.3. For peak designation, see Fig. 2.

5–40 $\mu g\ l^{-1}$ for 2-NP and 4-NP and between 10–40 $\mu g\ l^{-1}$ for the rest of the compounds studied, with regression coefficients higher than 0.990. The repeatability and reproducibility of the method were determined with 20 ml of river water spiked with 10 $\mu g\ l^{-1}$. The R.S.D.s ($n=4$) found were between 1.8–7.8% and 1.5–8.5%, respectively. The limits of detection calculated, as mentioned above, were between 1 $\mu g\ l^{-1}$ for 2-NP and 4-NP and 3 $\mu g\ l^{-1}$ for 2,4-DNP. Fig. 5 shows the chromatograms for 20 ml of Pisuerga river water and the same sample spiked with 5 $\mu g\ l^{-1}$ of 2-NP and 4-NP and 10 $\mu g\ l^{-1}$ of the rest of the compounds studied. One peak appeared at the same retention time as 2-NP but it could not be identified as a phenolic compound when compared with the UV spectra.

4. Conclusion

SFC separated five phenolic compunds, including phenol, in less than 6 min with good resolution for all compounds. In comparison to the coupling of SPE to RPLC, the on-line coupling of SPE to SFC only involves the additional step of drying the precolumn. This step was optimized for the different sorbents tested (C_{18}, PLRP-S and ENVI-Chrom P) and of these, PLRP-S was selected because the peaks were not distorted. Under the optimum conditions found, the method detected phenol and nitrophenols at concentration levels of between 0.2 and 1 $\mu g\ l^{-1}$ for tap water and between 2 and 6 $\mu g\ l^{-1}$ for river water. The R.S.D.s ($n=4$) were lower than 10% in both samples. Furthermore, the method enabled phenol to be determined with no matrix interference, which is a drawback when phenol is determined by SPE on-line coupled to RPLC with a UV detector.

Acknowledgments

We would like to thank Fundación Domingo Martínez for financial support given.

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