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Journal of Chromatography A, 988 (2003) 145–149
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Determination of major phenolic compounds in water by reversed-phase liquid chromatography after pre-column derivatization with benzoyl chloride

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Received 23 July 2002; received in revised form 10 December 2002; accepted 10 December 2002

Abstract

A simple reversed-phase LC method capable of detecting ng/ml quantities of phenolic compounds in water is described. Pre-column derivatization with benzoyl chloride is used for the separation and determination *o*-cresol, *m*-cresol, *p*-cresol, phenol, resorcinol, catechol and hydroquinone in water. The benzoyl derivatives formed within in 15 min, were extracted with dietyl ether, and then analyzed by liquid chromatography with UV detection at 232 nm. With a mobile phase of acetonitrile–tetrahydrofuran–water (54:6:40, v/v) the seven derivatives were eluted in 15 min. The detection limits were between 0.05 and 0.50 ng/ml for 50 ml of a standard water sample. The method was applied to the analysis of phenols in wine and river water. The recovery of the derivatives from pure water was 81–94% with relative standard deviations of 2.5–5.0%.

Keywords: Derivatization, LC; Phenols; Benzoyl chloride

1. Introduction

Phenols may occur in the aquatic environment as a result of their widespread use in numerous commercial products including pesticides, wood preservatives, dyes and synthetic intermediates. Phenols are also widely used in the chemical industry. The determination of phenols in the aquatic environment is important due to their toxicity even below mg l^{-1} levels [1]. Because of the large number of phenols and their high degree of toxicity increasing emphasis is placed on the availability of effective and sensitive determination techniques that facilitate the rapid recovery of a number of phenols from complex sample matrices at low concentrations [2,3]. Direct quantitative determination of phenols at mg l^{-1} levels has been performed using both normal-phase chromatography and absorbance detection [4], or reversed-phase chromatography with absorbance [5–7] or fluorescence detection [8]. Due to the low sensitivity of the detectors, these methods require large sample volumes when used for trace analysis. Derivatization is time-consuming, but the derivatives can sometimes be determined at more selective

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0021-9673/02/$ – see front matter
doi:10.1016/S0021-9673(02)02056-3

wavelengths and may be easier to analyse by LC because of higher retention or better peak shapes. Therefore, to improve the sensitivity and selectivity, detection techniques based on both post- [9,10] and pre-column [11,12] derivatization have been applied. Phenols at ng l^{-1} levels can be detected electrochemically but electrochemical detection is not widely applied and does not allow the use of all usual LC solvents [13].

The present study describes a selective and sensitive method for determination of phenols in water based on the reaction between phenols and benzoyl chloride. The extraction of phenols from water is a continuing problem. To increase the recovery, solid-phase extraction [14,15] or extraction after derivatization [3,5] (as in the form of ion-pair complexes) have been employed. The extraction of phenols in the form of their benzoyl chloride derivatives was used by us to improve their recovery. The UV spectra of the benzoyl chloride derivatives exhibit intense maxima at 232 nm in 40% aqueous acetonitrile. Consequently, it was our aim to devise a simple pre-column derivatization plus reversed-phase LC procedure for the determination of seven common phenols in water at ng ml^{-1} levels.

2. Experimental

2.1. Reagents and chemicals

Reference samples of the phenols and the benzoyl chloride were purchased from Aldrich. Sodium hydroxide, dietyl ether, acetonitrile and tetrahydrofuran was purchased from Merck. All other reagents were of analytical reagent grade and were used without further purification. Individual stock standard solutions were prepared by dissolving 100 mg of a phenol in 50 ml of deionized water. Working standard solutions were prepared by diluting the stock solution with deionized water. The stock solutions were stable for up to 1 week when stored in the dark at room temperature. Ultrapure Milli-Q water (Millipore) was used for the preparation of solutions.

The river water samples were collected (in 1-l dark colored glass bottles) from the Mert river (Samsun, Turkey) which flows near a garbage area. The bottles were previously washed with 0.2 mol l^{-1} HCl and repeatedly rinsed with deionized water. During sampling, the bottles were rinsed twice with the sample water, then filled and tightly capped. Wine samples were purchased from a grocery shop in Samsun, Turkey and were directly used. Samples were filtered through Millipore membrane filters (0.45-μm pore size, Millipore, Bedford, USA) and stored at 4 °C in a refrigerator.

2.2. Apparatus

LC analysis of benzoyl chloride derivatives of the phenols was performed with a Spectra SYSTEM (P4000) liquid chromatograph equipped with a Rheodyne injection valve provided with a sample loop of 20 μl, and a Spectra SYSTEM (UV 3000 HR) detector set at 232 nm. The output of the detector was monitored with a computer (IPX Spectra SYSTEM SN 4000) which contained the PC 1000 software computer program. Absorbance measurements for phenol derivatives were performed using a UV–visible spectrophotometer (Unicam UV/VIS, Australia). For pH measurements, a pH meter (Jenway model 3040 Ion Analyser) with combined glass-calomel electrode was used. The mobile phase and samples were filtered through a Millipore membrane filter. A Lichrosorb RP-C_8 (250×4.6 mm I.D., 5-μm particle size) column was used with a Lichrosorb RP-C_{18} guard column.

2.3. Derivatization and extraction procedure

A 50-ml sample of deionized water was spiked with a mixture of seven phenolic compounds to obtain individual final concentrations of 80 ng ml^{-1}, and mixed with a magnetic stirrer to ensure homogeneity. The water sample was alkalinized to pH 13 using concentrated sodium hydroxide which was added dropwise. The water sample was then derivatized using 1 ml of benzoyl chloride and was shaken vigorously for 15 min at room temperature. Excess benzoyl chloride was crystallized and the residue was filtered. Next the filtered sample was extracted with diethyl ether. The samples were extracted with 1×10 ml and 2×5 ml of diethyl ether. The water layer was saved and sodium chloride (2.9 g) added to increase the ionic strength and promote the

transfer of the phenol derivatives into the organic extract. The extraction was repeated with an additional 5 ml diethyl ether. For all extractions, separating funnels were shaken for 2 min and the phases were allowed to separate for 5 min. The modified and unmodified extracts were then combined and evaporated to dryness at 30 °C in a water bath. The precipitates were redissolved in 1 ml of mobile phase and 20 μl of each sample was injected onto the LC column.

3. Results and discussion

3.1. LC separation and analytical data

The LC separation of the benzoyl chloride derivatives of the phenols was studied by changing the acetonitrile–THF–water composition of the mobile phase. Good separations were obtained with acetonitrile–THF–water 54:6:40 (v/v). The presence of the less polar tetrahydrofuran was necessary to increase peak resolution in a short run time of ~15 min (flow-rate, 1 ml min^{-1}). The benzoyl chloride derivatives of phenol, *p*-cresol, resorcinol, hydroquinone and catechol were completely separated from each other under these conditions, but the *o*-cresol and *m*-cresol derivatives coeluted. Table 1 summarizes relevant analytical data for the phenol derivatives. The phenols were determined in the range 4–320 ng ml^{-1} with relative standard deviations of 1.0–5.5% and excellent linearity was obtained. The detection limits of these phenols in water were between 0.05 and 0.50 ng ml^{-1} for a 50-ml standard water sample.

3.2. Reaction conditions

As the derivatization reaction of phenolic compounds with benzoyl chloride proceeded in a basic medium, the effect of the pH in the presence of sodium hydroxide was examined using a standard solution of the phenols at a concentration of 80 ng ml^{-1}. The peak heights of all compounds reached maxima at pH 12–14. Temperatures of 20, 30 and 50 °C were tested. All peak heights reached a maximum after a reaction time of 15 min at all temperatures. Consequently, all further experiments were carried out at pH 13 and room temperature. The efficiency of the conversion of the phenols into the derivatives under the above conditions was not examined.

3.3. Recovery

Extraction of phenolic compounds was first examined by using the solvents hexane, diethyl ether and dichloromethane after the derivatization reaction. Diethyl ether was chosen as the best among them. For comparison, conventional liquid–liquid extraction with dichloromethane [16] prior to the derivatization reaction was also examined. Extraction of phenol derivatives with diethyl ether resulted a substantial improvement enabling a high recovery for the phenolic compounds from standard water sample when compared to conventional liquid–liquid extraction.

Table 1
Analytical data for the test compounds[a]

Analyte	t_R (min)	LOD[b] (ng/ml)	$y = ax + b$	r
Phenol	6.8	0.05	$y = 0.0003x + 0.0011$	0.98
Resorcinol	10.0	0.15	$y = 0.0003x + 0.0009$	0.99
p-Cresol	8.2	0.25	$y = 0.0003x + 0.0006$	0.99
m-Cresol	9.0	0.30	$y = 0.0003x + 0.0004$	0.98
o-Cresol	9.0	0.25	$y = 0.0002x + 0.0004$	0.99
Hydroquinone	13.8	0.45	$y = 0.0002x + 0.0002$	0.99
Catechol	14.8	0.50	$y = 0.0002x + 0.0001$	0.99

[a] For experimental conditions, see text.
[b] Defined as three times signal-to-noise ratio.

Table 2
Recoveries of phenols from standard water (after or prior to derivatization) and 50 ml river water (recovery(%)±RSD(%))

Analyte	Extraction after derivatization[a]	Liquid–liquid extraction prior to derivatization[b]	Phenols extracted from 50 ml of river water[c]
Phenol	95±4.0	72±5.0	94±3.0
Resorcinol	87±3.0	67±4.5	87±2.5
p-Cresol	86±3.5	65±3.5	86±3.5
m-Cresol	84±2.5	68±4.0	84±3.0
o-Cresol	81±4.0	57±5.0	81±4.0
Hydroquinone	91±5.0	62±5.0	91±5.0
Catechol	85±4.5	52±5.5	85±3.5

[a] Spiking at 40 and 160 ng/ml ($n=4$).
[b] Spiking at 80 ng/ml ($n=4$).
[c] Added 250 ng ($n=4$).

The efficiency of the extraction procedure and the recovery of phenols from 50 ml of river water are shown in Table 2. The quantities of the phenols were spiked to a concentration of 5.0 ng ml^{-1}, and over 81–94% of the phenols was recovered from river water with relative standard deviations of 2.5–5.0%.

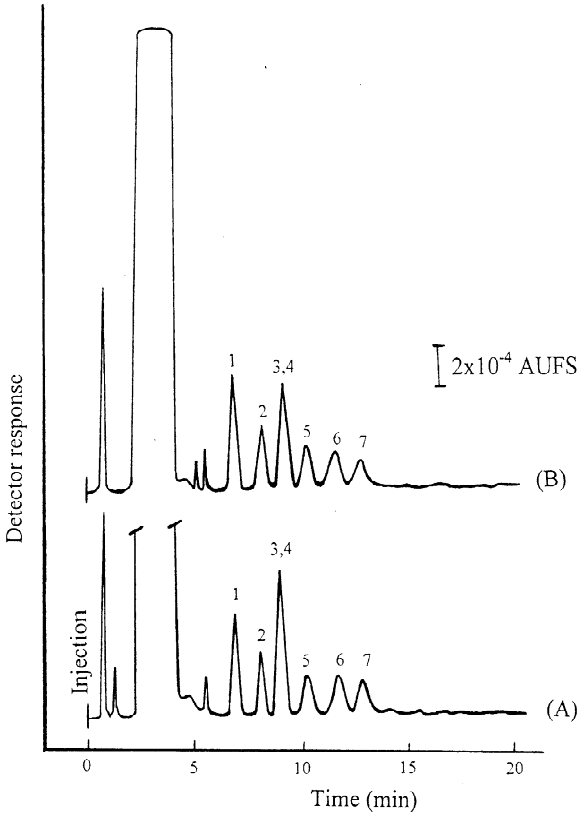

Fig. 1. Reversed-phase LC–UV chromatograms of benzoyl chloride derivatives of phenols obtained from 50 ml of (A) river water and (B) wine. For conditions and peak assignment, see text and Table 1.

3.4. Applications

A 50-ml sample of filtered river water or wine was spiked with 5 ng ml^{-1} of each phenol, brought to pH 13, derivatized and extracted as described above. Typical chromatograms are shown in Fig. 1A and B for spiked and Fig. 2A and B for non-spiked samples, respectively. The peaks due to the benzoyl derivatives of phenol, *o*- and *m*-cresol and hydroquinone in river water, and phenol in wine were assigned by comparing the retention times with those of spiked standard water samples. Obviously, low ng ml^{-1} levels of phenols can be successfully determined in such samples. The river water peaks due to phenol, *o*- and *m*-cresol and hydroquinone corresponded to 3.6, 2.2 and 7.6 ng ml^{-1}, respectively. Phenol peak in the wine sample corresponded to 2.1 ng ml^{-1}.

4. Conclusions

Derivatization of polar phenols with subsequent extraction of the derivatives into diethyl ether and LC–UV analysis is a simple and straightforward procedure to determine such compounds at the trace level in aqueous samples. Admittedly, because some of the analytes coelute, and further coelution with other (derivatized) phenols, etc., can not be ex-

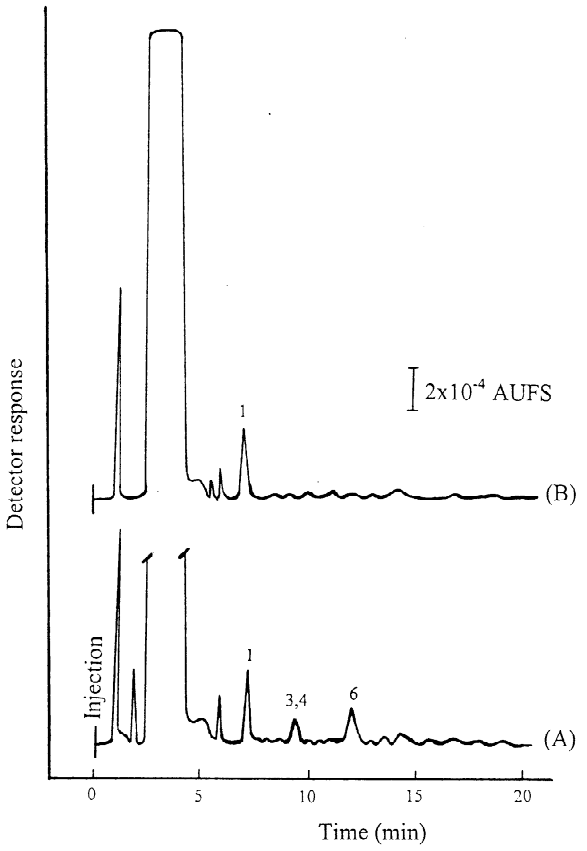

Fig. 2. Reversed-phase LC–UV chromatograms of benzoyl chloride derivatives of phenols obtained from 50 ml of (A) river water and (B) wine samples spiked at the individual level of 5 ng ml^{-1}. For conditions and peak assignment, see Fig. 1.

cluded, the procedure is primarily useful for screening purposes.

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