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Gas chromatographic determination of phenol compounds with automatic continuous extraction and derivatization

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ABSTRACT

Two gas chromatographic procedures for the determination of a variety of substituted phenols in water samples are described. The phenols were extracted or extracted–derivatized by using a continuous liquid–liquid extraction–derivatizaton system and quantified with flame ionization detection. Ethyl acetate was found to be the most efficient solvent for phenols whereas *n*-hexane yielded essentially the same recoveries for derivatized phenols. Between 0.1 and 300 mg/l of the different phenols can be detected with a relative standard deviation 1.1 and 3.7%. The acetate esters of six phenols were formed by the direct addition of acetic anhydride to the organic extractant. The stable acetate esters thus formed were isolated by using a standard dimethyl polysiloxane gas chromatographic column.

INTRODUCTION

Phenol compounds enter the environment in various ways: directly, as industrial effluents, and indirectly, as conversion products from natural and synthetic chemicals, including pesticides. These compounds, and particularly chlorophenols, possess odour and taste-spoiling properties [1–4] which makes it desirable to establish their concentration levels in the environment. Methods for the determination of trace phenol pollutants in aqueous samples generally include an extraction and/or concentration step prior to their separation and quantification by gas chromatography. The concentration step in most commonly accomplished by direct solvent extraction [5–13] or by adsorption and subsequent elution [14–16]. Because of the high polarity and low vapour pressure of phenols, a derivatization step is often used in their analysis to improve the chromatographic performance and, occassionally, more efficient extraction from aqueous samples. Reagents such as 1-fluoro-2,4-dinitrobenzene [17,18], heptafluorobutyrylimidazole [19], diazomethane [20], silanizing agents [21–24], pentafluorobenzoyl derivatives [25,26] and acetic anhydride [26,27–33] have been employed for this purpose. However, derivatization has some drawbacks such as the additional errors that it introduces. Sterically hindered phenols may react incompletely and partial decomposition of derivatives may occur during their storage. Moreover, many reagents used for derivatization are toxic, carcinogenic or explosive [34,35].

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Flow-injection techniques have also been employed for the determination of phenols. Thus, phenol was analysed for in waters by using liquid dialysis for isolation prior to its photometric detection [36]. The standard photometric 4-aminoantipyrine method has been automated for the determination of pentachlorophenol from conventional and stopped-flow measurements [37]. Electrochemical techniques have also frequently been used with flow systems for the determination of phenolic compounds [38–40].

The only method using a modified steam-distillation extraction device for the continuous extraction and preconcentration of phenol, *o*-, *m*- and *p*-cresols and 2,6-, 2,5-, 3,5-, 2,3- and 3,4-xylenols was reported by Curvers *et al.* [41]. Subsequent analysis was carried out by gas chromatography on a instrument equipped with a glass capillary column coated with OV-1 with helium as carrier gas. The relative standard deviation was 3% for ethyl acetate extractant.

The aim of this study was to overcome the drawbacks posed by operations preceding sample introduction in gas chromatography (GC) (*e.g.*, decrease sample manipulations, prevent solvents and derivatizing reagents from coming into contact with air and the operator). This was accomplished by using a continuous liquid–liquid extractor allowing the simultaneous extraction and derivatization of various phenolic compounds. By optimizing all the experimental variables involved in the system a method was developed that combines the efficiency of extraction and the sensitivity and selectivity of GC. The performance of the continuous extraction–derivatization system was studied by using phenols, cresols and chlorophenols. The acetate esters of six phenol compounds were obtained by adding acetic anhydride to the *n*-hexane extractant used.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 5890 A gas chromatograph furnished with a flame ionization detector was used. Chromatographic assays were performed on a 10 m × 0.53 mm I.D. dimethyl polysiloxane (film thickness 2.65 μm) coated fused-silica column obtained from Hewlett-Packard (HP-1). Nitrogen was used as the carrier gas (44 ml/min). The temperature of the injector was 150°C and the detector was kept at 250°C. The oven was kept at either 60°C for 2 min and then increased at 10°C/min to 150°C (2 min), or at 45°C for 5 min and then increased at 5°C/min to 150°C (2 min) for separation of phenols or acetate phenols, respectively. The injected sample volume was 2 μl and peak areas were measured by using a Hewlett-Packard 3392 A integrator. The flow extraction–derivatization system consisted of a peristaltic pump (Gilson Minipuls-2), an A-10T solvent segmenter (Tecator) and a custom-made phase separator with a Fluoropore membrane (1.0 μm pore size, FALP; Millipore) as described elsewhere [42]. Poly(vinyl chloride) and Solvaflex pumping tubes for aqueous and organic solutions, respectively, and Teflon tubing for the coils were used. A thermostated water-bath was also used.

Standards and reagents

Phenol, 3,4-dimethylphenol, 2-*tert.*-butylphenol, 2-*tert.*-butyl-4-methylphenol and naphthalene (internal standard) were obtained from Aldrich. All other phenols and reagents used (*o*-cresol, *m*-cresol, 2-chlorophenol, 4-chlorophenol, acetic anhydride, ethyl acetate, toluene, *n*-hexane, sodium hydrogencarbonate and sodium sulphate) were obtained from Merck.

Preparation of samples and reagents solutions

Stock standard solutions of phenol, 3,4-dimethylphenol, 2-*tert*.-butylphenol, 2-*tert*.-butyl-4-methylphenol, *o*- and *m*-cresol and 2- and 4-chlorophenol were prepared at a concentration of 1 g/l in 95% ethanol and stored in glass-stoppered bottles at 4°C.

For extraction, appropriate amounts of each stock solution were further diluted with distilled water to prepare 25 ml of solutions containing between 0.1 and 300 mg/l of each phenol (phenol, 3,4-dimethylphenol, 2-*tert*.-butylphenol and 2-*tert*.-butyl-4-methylphenol); the extractant consisted ethyl acetate containing 300 mg/l of naphthalene as internal standard.

For extraction and derivatization, appropriate amounts of each stock solution (phenol, 3,4-dimethylphenol, *o*-cresol, *m*-cresol, 2-chlorophenol and 4-chlorophenol) and 0.5 ml 2 *M* sodium hydrogencarbonate were added to 25-ml calibrated flasks and diluted with distilled water to prepare solutions containing 0.2–300 mg/l of each phenol compound in 0.04 *M* sodium hydrogencarbonate (pH 8.5). The extractant was *n*-hexane containing 300 mg/l of naphthalene (internal standard) and 6% (v/v) acetic anhydride (derivatization reagent).

Procedure

The manifold employed in depicted in Fig. 1. The sample solution was pumped continuously into the system and merged with a stream of ethyl acetate (for extraction only) or *n*-hexane (for simultaneous extraction and derivatization). The extraction (and derivatization) process took place in the extraction coil and the organic phase was isolated in the membrane phase separator. The ethyl acetate or *n*-hexane extracts of underivatized phenols or acetate derivatives, respectively, was collected in a 4-ml glass vial, containing anhydrous sodium sulphate as a desiccant, from which 2-μl portions were extracted with syringes and introduced into the chromatograph. Although the acetate esters were volatile, nothing was lost in their transfer to the chromatograph.

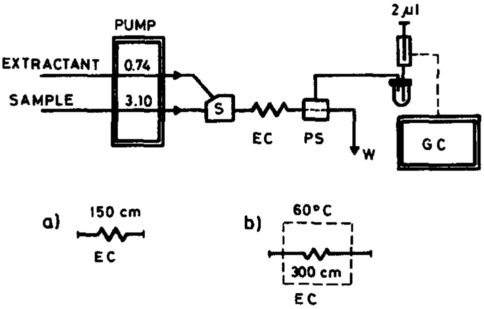

Fig. 1. Manifold for the chromatographic determination of underivatized or derivatized phenols. All tubes are made of Teflon (0.5 mm I.D.). S, solvent segmenter; EC, extraction coil, (a) for extraction and (b) for extraction and derivatization; PS, phase separator; W, waste. Flow-rates are given in ml/min.

TABLE I

RECOVERY (%) OF PHENOLS

Extractant: (A) for underivatized phenols; (B) extractant containing 6% (v/v) acetic anhydride for derivatization.

Compound	Ethyl acetate		Toluene		*n*-Hexane	
	A	B	A	B	A	B
Phenol	65.1	60.4	39.4	40.1	8.1	70.2
3,4-Dimethylphenol	76.2	70.2	80.1	75.1	18.3	75.4
2-*tert.*-Butylphenol	78.0	–	80.0	–	75.0	–
2-*tert.*-Butyl-4-methylphenol	56.9	–	55.6	–	61.2	–
o-Cresol	–	66.2	–	70.2	–	80.1
m-Cresol	–	58.4	–	76.4	–	78.9
2-Chlorophenol	–	65.1	–	66.2	–	69.5
4-Chlorophenol	–	55.8	–	70.5	–	77.8

RESULTS AND DISCUSSION

Ethyl acetate was found to be the most efficient extraction solvent for underivatized phenols, followed by toluene and *n*-hexane. Simultaneous acylation with acetic anhydride and extraction with *n*-hexane was comparable to the use of ethyl acetate alone in efficiency (Table I). The recoveries of phenol compounds was never higher than 80% because the extraction efficiencies in continuous extraction systems usually range between 70 and 80%, as chemical equilibrium is never reached; however, the recoveries were always reproducible. The recoveries listed in Table I were calculated by dividing the peak areas obtained in the continuous extraction mode into the corresponding peak area obtained by dissolving each phenol in its respective solvent at the same concentration. The conversion of the phenols to their corresponding esters yielded better GC peak symmetry than underivatized compounds and also allowed phenols, cresols and chlorophenols to be sequentially isolated (the bands of the underivatized phenols are usually overlapped).

Continuous extraction of underivatized phenols

Optimization of the method. For this study, a sample solution containing 30 mg/l of each phenol compound (phenol, 3,4-dimethylphenol, 2-*tert.*-butylphenol and 2-*tert.*-butyl-4-methylphenol) was prepared in distilled water. The extractant was ethyl acetate containing 300 mg/l of naphthalene as internal standard.

The effect of the sample pH was studied in the range 2–10; it was found to have no effect on the signals in the range 2–8. Above pH 8, the signals decreased owing to the formation of the corresponding phenolates, which decreased the phenol concentration. The ionic strength, adjusted with potassium nitrate, did not affect the signal up to 1 *M*.

The flow-rates of the aqueous and organic phases were also optimized. Fig. 2A shows the effect of the sample flow-rate at a constant flow-rate of ethyl acetate. Higher sample flow-rates resulted in greater peak areas owing to the increased preconcentra-

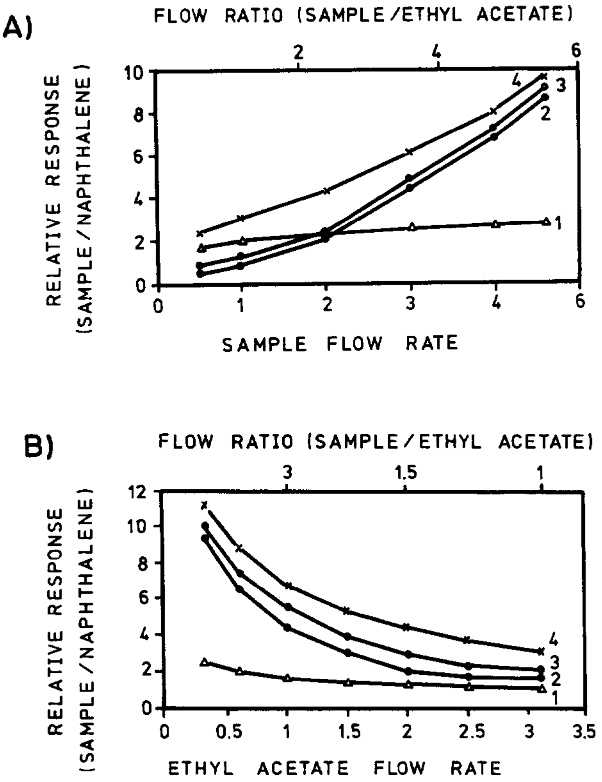

Fig. 2. Effect of (A) sample flow-rate (organic phase flow-rate = 0.84 ml/min) and (B) organic flow-rate (sample flow-rate = 3.1 ml/min). 1 = Phenol; 2 = 2-*tert*.-butylphenol; 3 = 2-*tert*.-butyl-4-methylphenol; 4 = 3,4-dimethylphenol. For GC conditions, see text.

tion ratio. The phenol signals remained virtually constant because of a potential mutual compensation of factors: increased signal from an increased preconcentration factor and decreased signal from decreased contact time between phases resulting from a decreased residence time of the analyte arising from increased flow-rates. Fig. 2B shows the variation of the signals as a function of the flow-rate of the organic phase. As expected, the peak area increased with decreasing organic phase flow-rate. Again, the phenol was the compound least affected by this variable. A sample flow-rate of 3.1 ml/min and an ethyl acetate flow-rate of 0.74 ml/min were chosen, taking into account the mutual influence of reproducibility, concentration ratio and sampling frequency. The influence of the residence time was also studied by varying the length of the extraction coil between 50 and 300 cm; the peak area was not affected by this variable. A length of 150 cm was therefore selected, which yielded a residence time of 5 s.

Calibration graphs for the underivatized phenols. The calibration graphs obtained by plotting the ratio of analyte peak area/internal standard peak area *versus* the analyte concentration in the aqueous medium were reproducible and linear over the range tested (0.1–300 mg/l). The features of these graphs and those of the analytical

TABLE II

FEATURES OF THE CALIBRATION GRAPHS AND DETERMINATION OF UNDERIVATIZED PHENOLS

Compound	Regression equation[a]	r[b]	Linear range (mg/l)	Detection limit (mg/l)	Relative standard deviation (%)
Phenol	$A = 7.80 \cdot 10^{-3} X + 2.23 \cdot 10^{-4}$	0.9992	0.2–3	0.15	2.81
3,4-Dimethylphenol	$A = 8.66 \cdot 10^{-3} X + 1.19 \cdot 10^{-3}$	0.9993	0.3–3	0.20	3.56
2-*tert.*-Butylphenol	$A = 9.16 \cdot 10^{-3} X + 1.21 \cdot 10^{-3}$	0.9994	0.1–3	0.10	3.53
2-*tert.*-Butyl-4-methylphenol	$A = 6.82 \cdot 10^{-3} X - 1.90 \cdot 10^{-4}$	0.9994	0.1–3	0.10	3.32
Phenol	$A = 6.58 \cdot 10^{-3} X + 2.20 \cdot 10^{-3}$	0.9996	3 –30		1.82
3,4-Dimethylphenol	$A = 6.81 \cdot 10^{-3} X + 5.46 \cdot 10^{-3}$	0.9996			2.24
2-*tert.*-Butylphenol	$A = 4.96 \cdot 10^{-3} X + 1.11 \cdot 10^{-2}$	0.9995			2.44
2-*tert.*-Butyl-4-methylphenol	$A = 5.42 \cdot 10^{-3} X + 5.06 \cdot 10^{-3}$	0.9995			2.62
Phenol	$A = 7.93 \cdot 10^{-3} X - 1.18 \cdot 10^{-2}$	0.9997	30 –300		1.11
3,4-Dimethylphenol	$A = 8.92 \cdot 10^{-3} X - 2.52 \cdot 10^{-4}$	0.9997			1.55
2-*tert.*-Butylphenol	$A = 7.53 \cdot 10^{-3} X - 8.63 \cdot 10^{-4}$	0.9997			1.75
2-*tert.*-Butyl-4-methylphenol	$A = 7.44 \cdot 10^{-3} X - 7.88 \cdot 10^{-3}$	0.9996			2.05

[a] A = analyte peak area/internal standard peak area ratio; X = concentration in mg/l.
[b] r = Correlation coefficient.

procedure are summarized in Table II at three integrator sensitivities. The practical detection limit was calculated as the concentration yielding the minimum detectable signal in the chromatogram. The relative standard deviation was checked on eleven samples containing 1, 15 or 35 mg/l of each phenolic compound in the linear range assayed, 0.2–3, 3–30 and 30–300 mg/l, respectively.

Simultaneous extraction and derivatization of phenol compounds

The proposed method for the continuous extraction and GC determination of phenolic compounds allows the isolation and identification of the four phenols assayed; however, if the samples also contain cresols and/or chlorophenols, their separation is not sequential and the chromatogram contains overlapped peaks. The conversion of the phenols, cresols and chlorophenols to their corresponding esters simultaneously with extraction into *n*-hexane ostensibly improved the chromatographic resolution.

Optimization of the method. First we studied the optimum pH for the derivatization reaction. For this purpose, two aqueous phenolic solutions of 30 mg/l of each phenol (phenol, 3,4-dimethylphenol, *o*-cresol, *m*-cresol, 2-chlorophenol and 4-chlorophenol) at a final pH of 3 or 8.5 were prepared. The extractant was *n*-hexane containing 300 mg/l of naphthalene and 6% acetic anhydride.

The yields of the acetate derivatives of the phenols obtained from an acidified water sample (pH 3) was lower than those obtained with an alkaline water sample (pH 8.5), thus indicating that acylation with acetic anhydride proceeded far more rapidly on the phenolate ions than on the undissociated compounds. The acetylation yields were not influenced by the hydrogencarbonate concentration over the range 0.03–0.05 *M*. For continuous derivatization–extraction, the aqueous samples were prepared in 0.04 *M* sodium hydrogencarbonate solution (pH 8.5). The influence of the ionic strength on the aqueous samples (adjusted with potassium nitrate) was also tested; it had no effect up to a concentration of 1 *M* (similarly to the extraction of the underivatized phenols).

To study the influence of the concentration of acetic anhydride on the yield of acetate derivatives, several solutions in the range 0.2–7% in *n*-hexane were prepared. A 4% solution of the organic reagent was found to be sufficient for maximum response; lower concentrations resulted in incomplete derivatization and thus in the co-occurrence of peaks from the underivatized and derivatized phenols in the chromatogram. The relative response (sample/naphthalene) remained constant at higher concentrations. A 6% content of acetic anhydride in *n*-hexane was selected as extractant for routine analyses.

In manual procedures, the derivatization of phenolic compounds is favoured by heating [30] as it is in the continuous mode. The effect of temperature was studied in the range 20–75°C; the yield of the derivatization reaction was optimum above 40°C, below which the chromatogram obtained showed peaks corresponding to the underivatized phenols and esters. Fig. 3 shows the influence of temperature on the derivatization process for four phenolic compounds; the cresols assayed behaved similarly. The extraction coil was kept at 60°C by using a thermostated water-bath.

The influence of the extraction–derivatization coil length was investigated in the range 50–500 cm. Above 150 cm the signal remained virtually constant. There is a difference with the continuous method with extraction only (where this variable has no effect) as it requires some time for the esters to be formed. A thermostated (60°C) reaction coil of length 300 cm, which resulted in a residence time of 9 s, was chosen.

Determination of phenols, cresols and chlorophenols. The GC separation of the underivatized and acetylated phenols is illustrated in Fig. 4A and B, respectively. As can be seen, the peaks of the underivatized phenols and chlorophenols extracted into *n*-hexane are completely overlapped. In the manual preparation of esters of phenols, several workers have claimed [43] that the excess of anhydride must be removed prior to GC. As shown in Fig. 4B, excess of acetic anhydride did not interfere with the quantification of the derivatives, and thus need not be removed.

The calibration graphs for the acetic anhydride derivatives of phenol, 3,4-di-

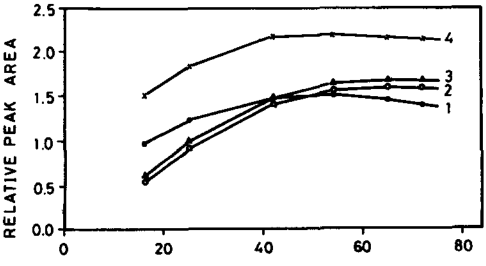

Fig. 3. Influence of temperature on the derivatization reaction. 1 = 2-Chlorophenyl acetate; 2 = 3,4-dimethylphenyl acetate; 3 = phenyl acetate; 4 = 4-chlorophenyl acetate. Extractant: *n*-hexane containing 6% acetic anhydride.

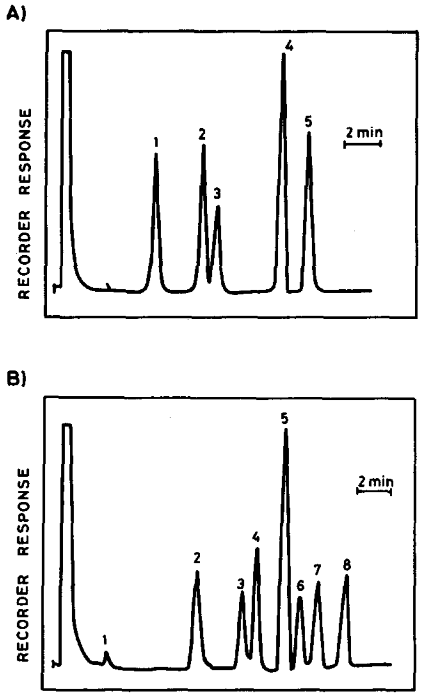

Fig. 4. (A) Gas chromatogram of underivatized phenols. Peaks: 1 = phenol + *o*-cresol; 2 = *m*-cresol; 3 = 2-chlorophenol; 4 = naphthalene; 5 = 3,4-dimethylphenol + 4-chlorophenol. (B) Gas chromatogram of acetate derivatives. Peaks: 1 = acetic anhydride; 2 = phenyl acetate; 3 = *o*-cresyl acetate; 4 = *m*-cresyl acetate; 5 = naphthalene; 6 = 2-chlorophenyl acetate; 7 = 4-chlorophenyl acetate; 8 = 3,4-dimethylphenyl acetate. The extractant was *n*-hexane and the column temperature was 45°C for 5 min, increased to 150°C at 5°C/min and held at 150°C for 2 min.

methylphenol, *o*-cresol, *m*-cresol, 2-chlorophenol and 4-chlorophenol were linear at concentrations between 5 and 7500 μg in 25 ml of distilled water at pH 8.5. The relative standard deviation ranged between 1.5 and 3.5%.

CONCLUSIONS

Phenols are usually derivatized in order to improve their GC features or enhance their extractability from aqueous solutions. However, the manual procedures used for this purpose are lengthy and require several steps, which gives rise to losses and low reproducibility; on the other hand, the method proposed here permits the direct acetylation of the aqueous test solution simultaneously with the extraction in a single step.

The two continuous methods proposed here for the extraction and extraction–derivatization of phenols feature all the inherent advantages of automatic methods (*viz.*, low sample and reagent consumption, minimal manipulation and contact with the reagents, accurate and reproducible results, high sampling frequencies, etc.). These automatic methods allow the derivatization and extraction of phenol compounds with a sampling frequency of 35 ± 5 h^{-1}. Such a high rate, however, is limited by the oven temperature programming.

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