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GAS CHROMATOGRAPHIC ANALYSIS OF TRACE PHENOLS BY DIRECT ACETYLATION IN AQUEOUS SOLUTION

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SUMMARY

Acetate esters of six phenolics were formed by the direct addition of 500 μl acetic anhydride to 250 ml of a dilute aqueous phenolic solution containing 10 g sodium bicarbonate. In the concentration range of 0.08–0.24 μmoles/l, phenol, *o*-cresol, *m*-cresol, *p*-cresol, 2,4-dichlorophenol and 1-naphthol easily formed acetate esters which provided for improved gas chromatographic characteristics and virtually quantitative recovery from aqueous solution. On extraction with small volumes of methylene chloride, phenol could only be recovered to the extent of 28–41% in the 0.2–2.0 mg/l range. On the other hand, phenyl acetate, formed in water prior to extraction, was 100% recovered. The stable acetate esters can be analyzed using standard gas chromatographic columns such as OV-17 or OV-101 while phenolics generally require specially deactivated packings. On 1% SP-1240 DA all of the derivatives, with the exception of *m*- and *p*-cresyl acetate, could be separated.

INTRODUCTION

Effluent limitations are being established for a variety of toxic chemicals by the U.S. Environmental Protection Agency (EPA) and phenolic compounds are included on the list of priority pollutants[1]. Phenols may be derived from various sources including the petrochemical[2–4] and pulp and paper industries[5,6] as well as municipal wastes[7,8]. They are usually found in low concentrations (generally 75 μg/l or less) in river[9,10] and other water samples[11,12] but routine disinfection of drinking water by chlorination can give rise to chlorinated phenols[13]. These byproducts, in turn, may have an enhanced odor and taste potential as well as a reduced biodegradability relative to the parent phenol.

Methods for the determination of trace phenolic pollutants in aqueous samples generally include an extraction and/or concentration step although aqueous-injection gas–liquid chromatography (GLC) has been applied[3,14,15]. The concentration step is most commonly accomplished by direct solvent extraction[16,17] or by adsorption and subsequent elution from XAD resins[18–20] or charcoal (carbon)[19]. Quantitative extraction of phenolics is not often achieved by these methods although recovery may be

enhanced by prior acidification of the water sample. The adsorbents suffer an additional disadvantage in that they require thorough purification prior to use[18,19]. In our hands adsorption of phenols onto XAD-2 resins was found to be unsatisfactory. The recovery of phenol, *m*-cresol and 2,4-dichlorophenol at a concentration of 2.0 mg/l for each phenol was 44.3%, 80.6% and 88.3%, respectively.

The quantitative analysis of phenols has also been hampered by their poor GLC characteristics[21,22]. While a recent publication[1] of the EPA suggests the use of Tenax[23] for the GLC analysis of eleven phenols in water, SP-1240 DA[24] is an attractive alternative. This phosphoric acid deactivated polyester stationary phase provides greatly reduced tailing of GLC peaks and improved detector sensitivity. Alternatively, tailing may be minimized and sensitivity enhanced by derivatization of the phenolic group with various reagents including diazomethane[25], heptafluorobutyrylimidazole[26], trifluoroacetic anhydride[27] and silanizing reagents[28]. Derivatization with any of these reagents is possible, however, only after the phenols have been removed from the aqueous solution. The present study was initiated to determine whether an analytical method could be found which combined efficiency of extraction with sensitivity of detection and quantitation using a GLC method. For analyses, distilled water samples were spiked with low concentrations of representative phenols frequently isolated from environmental samples including phenol, *o*-cresol, *m*-cresol, *p*-cresol, 2,4-dichlorophenol and 1-naphthol (internal standard). The internal standard is itself a pollutant and can arise as the hydrolysis product of the pesticide Carbaryl[29].

EXPERIMENTAL

Apparatus

The gas chromatograph was a Perkin-Elmer Model 990 equipped with a flame-ionization detector.

Chromatographic conditions

The glass column (1.25 m × 4 mm I.D.) used was packed with 1% SP-1240 DA on Supelcoport (100–120 mesh) obtained from Supelco, (Bellefonte, Pa., U.S.A.). The column was conditioned at 200° for 24 h with a helium flow-rate of 60 ml/min. The operating conditions were: column temperature, 85–195° (8°/min); detector, 250°; injector, 250°; helium flow-rate, 60 ml/min.

The glass column and glass wool plugs were deactivated by silanization using a published procedure[30].

Reagents

Sodium bicarbonate, acetic anhydride, methylene chloride, phenol, *o*-cresol, *m*-cresol and 1-naphthol were obtained from Fisher Scientific (Fair Lawn, N.J., U.S.A.). The other phenols used for this study were: *p*-cresol (Eastman-Kodak, Rochester, N.Y., U.S.A.) and 2,4-dichlorophenol (Matheson, Coleman and Bell, Norwood, Ohio, U.S.A.). Naphthalene was obtained from Anachemia (Montreal, Canada).

Preparation of standard solutions

Standard stock solutions of phenol, *o*-cresol, *m*-cresol, *p*-cresol, 2,4-dichlorophenol and 1-naphthol were prepared at a concentration of 0.1 m*M* in 95% ethanol and stored in glass-stoppered bottles at 4°.

Preparation of test solutions

Appropriate amounts of each stock solution were further diluted with distilled water to prepare 500 ml test solutions containing 0.1 μmoles 1-naphthol and from 0.05–0.3 μmoles of each of the other phenols. The derivatization procedure was then performed in duplicate on 250-ml aliquots of each test solution. Naphthalene was used as internal standard when the extraction efficiency of phenol and phenyl acetate was compared.

Derivatization, extraction and concentration

Acetic anhydride esters of the phenols were prepared in a 500-ml separatory funnel as detailed in the following scheme:

Test solution (250 ml water) → Add 10 g $NaHCO_3$ (excess) → Add 500 μl acetic anhydride ↓ Extract with CH_2Cl_2 (2 × 10 ml) → Combine CH_2Cl_2 extracts Concentrate to 20 μl → GLC (1% SP-1240 DA)

After the dissolution of $NaHCO_3$ and addition of acetic anhydride, the separatory funnel was shaken vigorously until the evolution of carbon dioxide had ceased. This gave a solution with a final pH 8. Each extraction was accomplished by shaking with methylene chloride for 2.0 min and allowing the layers to separate completely. The combined total volume of 16.0–16.5 ml methylene chloride was then concentrated to 20 μl by means of a gentle stream of nitrogen. Although the acetate derivatives are volatile, insignificant amounts were lost during this concentration step.

Extraction of underivatized phenols

The aqueous phenolic solution ranging in concentration from 0.188–94 mg/l, was acidified with 6 *M* HCl to pH 3 and extracted with small volumes of methylene chloride.

RESULTS AND DISCUSSION

Gas–liquid chromatography

The GLC separation of the acetylated phenols is shown in Fig. 1. Peak 5 remains unidentified and occurs as an impurity in the distilled water used to make the test solutions. In the preparation of heptafluorobutyryl esters of phenols, Ehrsson *et al.*[31] reports that the excess anhydride must be removed prior to GLC. As shown in Fig. 1 excess acetic anhydride does not interfere with the quantitation of the derivatives and therefore need not be removed.

The gas chromatograms of phenol, *o*-cresol, *m*-cresol, *p*-cresol and their acetate derivatives on SP-1240 DA are shown in Fig. 2A and B, respectively. Derivatization

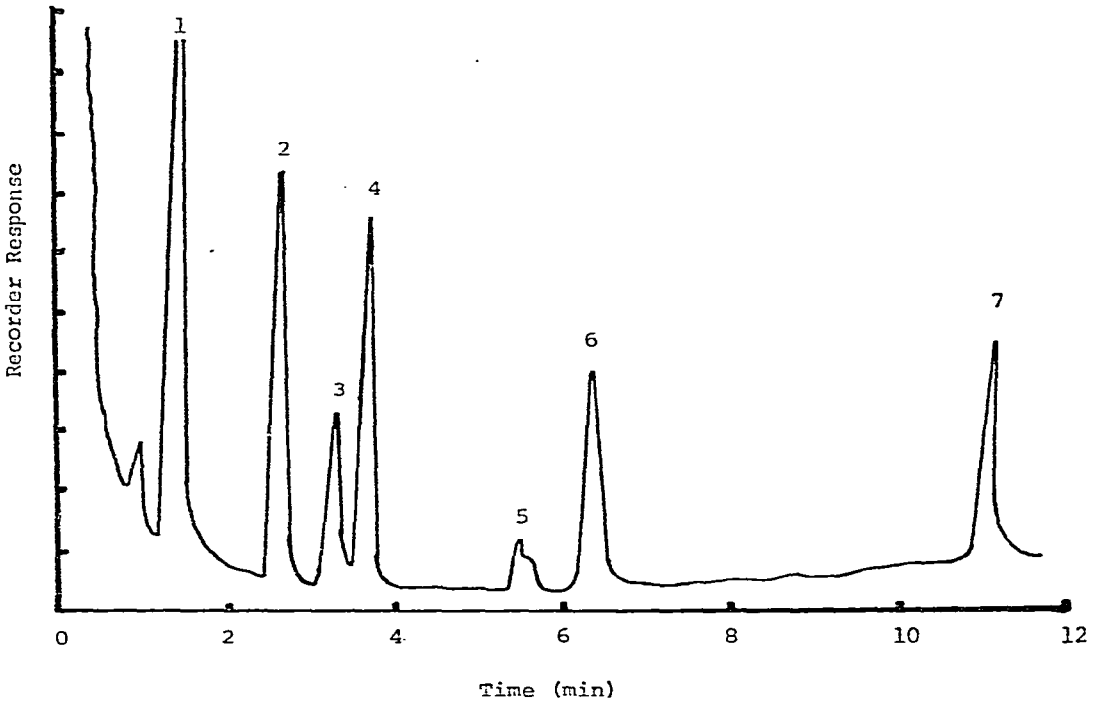

Fig. 1. Gas chromatogram of acetate esters. GLC conditions are given in the text. Peaks: 1 = acetic anhydride; 2 = phenyl acetate (3.8 nmoles); 3 = *o*-cresyl acetate (2.5 nmoles); 4 = *m*-cresyl acetate (3.8 nmoles); 5 = unidentified impurity; 6 = 2,4-dichlorophenyl acetate (3.0 nmoles); 7 = 1-naphthyl acetate (2.5 nmoles).

with acetic anhydride in aqueous solution is an efficient procedure as evidenced by the absence of unreacted phenols in Fig. 2B. It is also apparent that acetate esters have shorter retention times and better recorder response than the corresponding phenols. Although the separation of phenol and *o*-cresol is substantially improved the acetates of *m*- and *p*-cresol remain unresolved. Efforts to effect separation using OV-17, OV-330, XE-60, Carbowax, neopentyl glycol and various instrumental conditions were equally unsuccessful. The three cresol isomers can be separated on tris-(2,4-xylenyl)phosphate[32] or Carbopack C/0.1% SP-1000[33]. While Carbopack will also separate the acetate ester derivatives, a low temperature limit combined with long retention times makes this column undesirable.

Phenolic compounds can best be quantitated using acid deactivated columns such as SP-1240 DA which overcome peak asymmetry. The stable acetylated derivatives have good gas chromatographic properties and can be analyzed using SE-30, OV-17 or other untreated packings.

Derivatization and extraction

Lamparski and Nestrick[26] have reported extraction efficiencies of 70–85% for a variety of phenols in acidified water at the 50 μg/l level. However, the use of an equal volume of organic solvent to aqueous phase would be impractical for the extraction of large volumes of very dilute phenolic solutions. One objective of the present

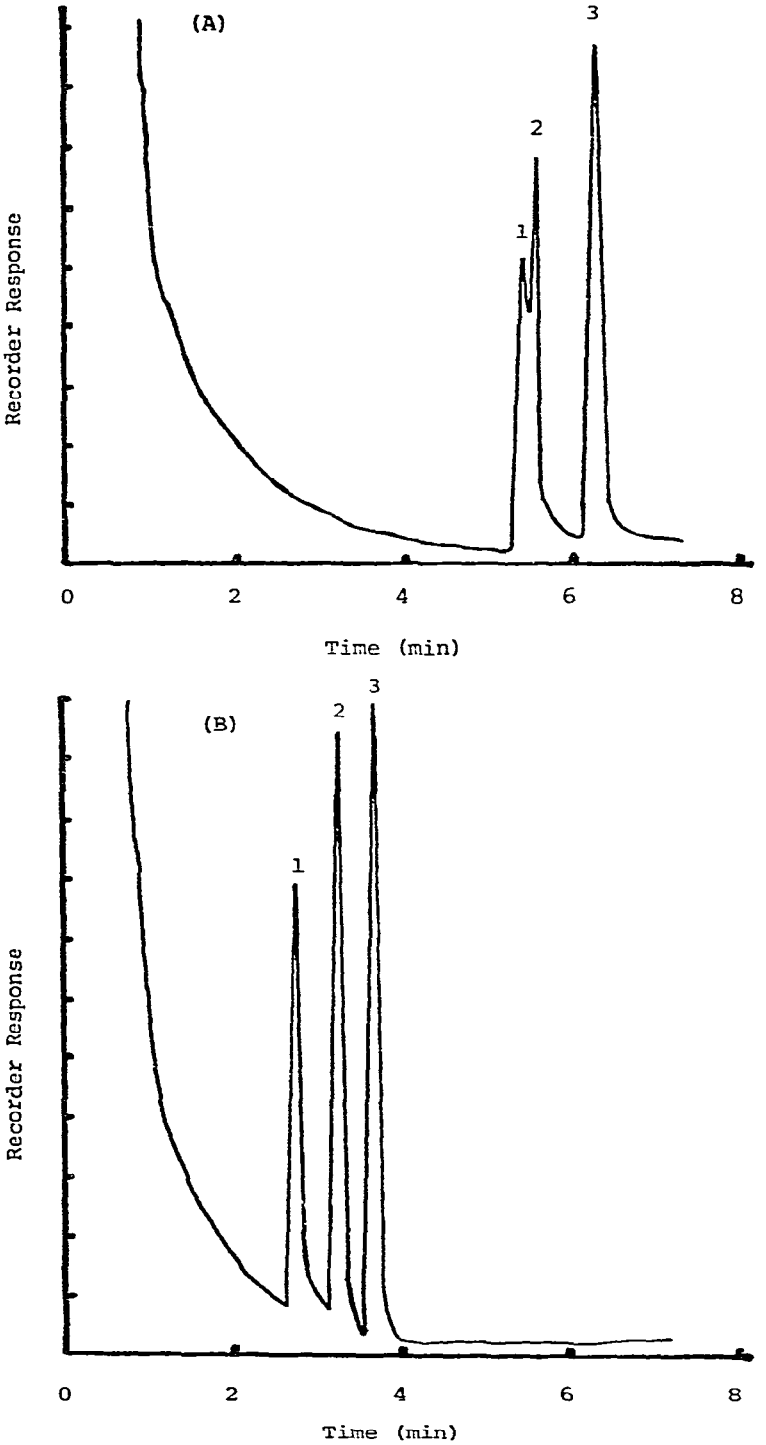

Fig. 2. Gas chromatograms of (A) underivatized phenols (500 ng of each). Peaks:1 = phenol; 2 = *o*-cresol; 3 = *m*-cresol + *p*-cresol. (B) Acetate derivatives (543 ng of each). Peaks: 1 = phenyl acetate; 2 = *o*-cresyl acetate; 3 = *m*-cresyl acetate + *p*-cresyl acetate. GLC conditions are given in the text.

study was to keep the volume of extracting solvent as low as possible and when this was done (Table I), efficiency of recovery was found, as expected, to be directly related to the concentration of the phenol in the aqueous solution. When, for example, 1.0 ml of water which contained 94 μg of phenol was extracted with 1.0 ml of methylene chloride, virtually all of the phenol entered the organic phase. However, when 10 ml of water containing 9.4 μg/ml of phenol was similarly extracted with 1.0 ml of solvent, only 51 % of the phenol was detected in the organic solution.

TABLE I

EFFECT OF CONCENTRATION AND SOLVENT:WATER RATIO ON THE EXTRACTION EFFICIENCY OF PHENOL AND PHENYL ACETATE

Concentration (mM)	*Water:CH_2Cl_2 ratio*	*Concentration (μg/ml)*		*Recovery (%)*	
		Phenol	*Phenyl acetate*	*Phenol**	*Phenyl acetate*
1.0	1:1	94	136	100	100
0.1	10:1	9.4	13.6	50.8	93.6
0.05	20:1	4.7	6.8	42.2	106.7
0.002	50:1	0.188	0.272	28.3	100.0

* A calibration curve similar to Fig. 3 was constructed to determine phenol recoveries using naphthalene as the internal standard.

The possibility of derivatizing the phenol in the aqueous phase was then explored. In their studies on biogenic amines Baker *et al.*[34], showed that very dilute aqueous solutions of 5-hydroxytryptamine, a phenolic amine, was both N- and O-acetylated when vigorously shaken with a one-tenth volume of acetic anhydride in the presence of excess sodium bicarbonate. The N,O-diacetylated product was readily extracted from the aqueous medium into ethyl acetate. This methodology was adapted to the present study.

The calibration graphs for acetic anhydride derivatives of phenol, *o*-cresol, *m*-cresol and 2,4-dichlorophenol are shown in Fig. 3. The internal standard (1-naphthol) was added to the water sample to be analyzed and carried through the entire derivatization, extraction, evaporation and chromatographic procedure. A good linearity was obtained for concentrations ranging from 0.025–0.15 μmoles in 250 ml distilled water.

The recovery of phenol from 250 ml of acidified water using small volumes of methylene chloride was found to be 28–41 % in the 0.2–2.0 mg/l concentration range. On the other hand, the recovery of phenol as phenyl acetate at the 0.2 mg/l level was found to be 100 %.The method outlined in the section *Derivatization, extraction and concentration* was applied to the analysis of four synthetic mixtures containing from 0.02–0.06 μmoles of phenols in 250 ml distilled water. The amount of phenols added and found by extraction are shown in Table II. Prior to extraction and derivatization 0.05 μmoles of 1-naphthol was added as internal standard to each aqueous solution. The recoveries ranged from 90–104 % and demonstrate that the method can accurately quantitate low concentrations of phenols in aqueous solution.

Low polarity polystyrene divinyl benzene resins such as XAD-2 are being used with increased frequency to extract trace organic compounds from water. The macroreticular resins most efficiently adsorb nonionic species while ionic compounds

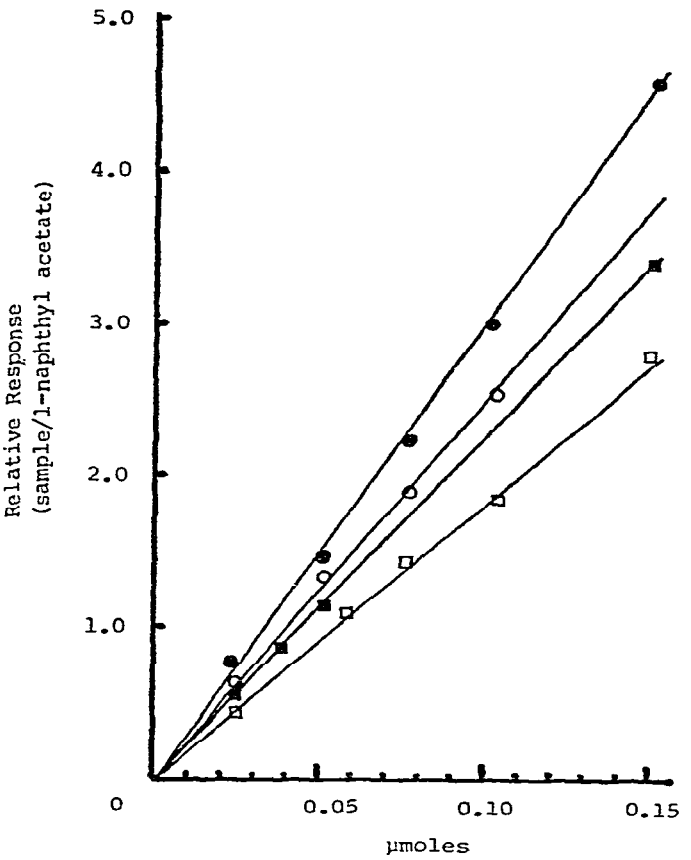

Fig. 3. Calibration graphs for acetate esters in the concentration range 0.025–0.15 µmoles in 250 ml distilled water: ●, Phenyl acetate; ○, *m*-cresyl acetate; ■, *o*-cresyl acetate; □, 2,4-dichlorophenyl acetate. GLC conditions are given in the text.

TABLE II

RECOVERY OF PHENOLS AS ACETATES FROM 250 ml AQUEOUS SOLUTION

	Phenols			
	Phenol	*o-Cresol*	*m-Cresol*	*2,4-Dichlorophenol*
Added (µmoles)	0.035	0.03	0.06	0.035
Found (µmoles)	0.035	0.029	0.056	0.032
Recovery (%)	100	97	93	92
Added (µmoles)	0.025	0.02	0.04	0.025
Found (µmoles)	0.026	0.019	0.041	0.0255
Recovery (%)	104	95	102	102
Added (µmoles)	0.06	0.05	0.02	0.055
Found (µmoles)	0.058	0.046	0.02	0.055
Recovery (%)	97	92	100	100
Added (µmoles)	0.02	0.035	0.035	0.02
Found (µmoles)	0.02	0.032	0.034	0.0205
Recovery (%)	100	90	97	102

prefer the aqueous phase. Van Rossum and Webb[19] reported recoveries of 14–46 and 33–69% for phenol and *p*-cresol, respectively at the 50 μg/l level. The authors stated that "significant amounts of phenols were found to pass through the column", *i.e.*, were not retained by the resin bed. A mini-column technique[20] gave 64% recovery of *o*-cresol at the 2–10 μg/l level and 40% at the 100 μg/l level from acidified water. Junk *et al.*[18], have reported recoveries from acidified water of 40%, 73% and 91% for phenol, *o*-cresol and 1-naphthol, respectively, in the 10–50 μg/l range. The corresponding recoveries in untreated water are 41%, 62% and 43%, respectively. The authors further report that the extraction efficiency of the resin is <100% for compounds capable of dissociating in water. These results suggest that since an aqueous acetylation procedure using acetic anhydride converts the partially dissociated, water soluble phenols into non-dissociated species the use of macroreticular resins would become more efficient.

The derivatization of phenols in aqueous media has been reported in the literature. Makita *et al.*[35] have prepared O-isobutyloxycarbonyl derivatives by the reaction of phenols with isobutyl chloroformate. However, acetic anhydride is more conveniently handled than is the chloroformate, a potent lacrimator. Krijgsman and van de Kamp[36] have reported the aqueous acetylation of chlorophenols using acetic anhydride. However, their procedure was lengthy and included a preliminary extraction of the phenols from the aqueous phase whereas our method describes the direct acetylation of the aqueous test solution.

CONCLUSION

In general, phenols are derivatized in order to improve their GLC characteristics or to enhance extractability from aqueous solution. The direct formation of acetate esters in large volumes of water not only produces stable GLC derivatives but also allows for near quantitative extraction of trace phenolics in the 8–40 μg/l range using small volumes of extraction solvent.

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