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SENSITIVE GAS CHROMATOGRAPHIC DETERMINATION OF PHENOLS AS BROMOPHENOLS USING ELECTRON CAPTURE DETECTION

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SUMMARY

Eight phenols, phenol, *o*-, *m*- and *p*-cresol, 2,3-, 2,5-, 3,4- and 3,5-xylene, were converted into the corresponding bromophenols by reaction with bromine. The optimum conditions for the bromophenol derivative formation of 10^{-8} mol of each phenol in 1 ml of ethanol were as follows: mol ratio (bromine/phenols), greater than 400; reaction temperature, room temperature; reaction time, 4 h. The minimum detectable amount of the bromophenols with an electron capture detector was about 0.01 ng, which is about 100 times less than the minimum detectable amount of the non-bromine-containing phenols.

INTRODUCTION

The sensitive detection of phenols remains a problem in toxicological and pesticidal analyses. Many workers have tried to solve this problem by derivatization of the phenols for detection with an electron capture detector (ECD) at sub-nanogram levels. Argauer¹ has reported a method using aqueous sodium hydroxide solution containing as little as 0.01 ppm of phenols with chloroacetic anhydride in benzene to prepare a relatively stable derivative which is sensitive to the ECD. Kawahara^{2,3} has reported a method for detecting phenols, mercaptans and organic acids as pentafluorobenzoyl ethers, thioethers and esters. Heeman and McCallum⁴ have reported a new method for the chromatographic detection of phenols as diethyl phenyl phosphate derivatives using a flame photometric detector (FPD). Chin *et al.*⁵ have reported a micro-determination of some phenols by mono- and polychloroacetylation followed by coulometric gas chromatography (GC). Cohen *et al.*⁶ have studied the trace detection of 40 phenols as their 2,4-dinitrophenyl ethers by GC-ECD. Davis⁷ has described a method for ECD micro-determination of phenols and carboxylic acids as pentafluorobenzoyl bromides, for which the minimum detectable amount for the standard solution is *ca.* 0.3 pmol. However, the separation of the corresponding derivatives of *o*-, *m*- and *p*-cresol

based on these derivatization techniques is difficult when a packed column employed.

In this study, in order to attain the complete separation and sensitive GC analysis of the phenols, especially of the isomers *o*-, *m*- and *p*-cresol, the phenols were converted into the corresponding bromophenols by reaction with bromine, and the bromophenols produced were detected by GC-ECD.

EXPERIMENTAL

Reagents

Phenol, *o*-ethylphenol, *p*-ethylphenol, 2,3-xyleneol, 2,4-xyleneol, 2,5-xyleneol, 3,4-xyleneol, 3,5-xyleneol, *o*-dinitrobenzene (used as an internal standard), ethanol and bromine were obtained from Wako (Osaka, Japan). *o*-Cresol, *m*-cresol, *p*-cresol and 2,6-xyleneol were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). All reagents used were guaranteed or reagent grade chemicals, and were used without further purification.

Preparation of bromophenols

All the bromophenols were made on a preparative scale as follows⁸. The brominating solution was prepared by dissolving 15 g of potassium bromide in 100 ml of water; 10 g of bromine were added. This solution was added slowly, with shaking, to a solution of 1 g of a phenol dissolved in 10 ml of water or ethanol. Just enough of the brominating solution was added to impart a yellow colour to the mixture. About 50 ml of water was then added, and the mixture was shaken vigorously. The precipitate of bromophenol produced was removed by filtration and washed with a dilute solution of sodium bisulphite until the yellow colour of remaining bromine disappeared, and dried over silica gel in a vacuum desiccator. The bromophenols obtained were sufficiently pure for GC analysis. However, 2,4-xyleneol, 2,6-xyleneol, *o*-ethylphenol and *p*-ethylphenol did not give the crystalline precipitates of the corresponding bromophenols in the reaction period.

All the bromophenols were prepared on a submicrogram scale as follows. 10 μ l of a solution of a phenol in ethanol (10^{-5} mol/10 ml) was added with a 100- μ l Termo microsyringe (MS-100) to 1 ml of ethanol, and immediately 1–400 μ l of a fresh bromine-ethanol solution (1% w/v) was added to the reaction mixture. After 4 h at room temperature, the excess of bromine was removed by nitrogen, bubbled throughout a flow-rate of 60 ml/min for 20 min. One microlitre of the sample solution was injected with a 10- μ l Hamilton microsyringe into the column and detected by an ECD. The reaction percentage was estimated from the amounts of the bromophenols produced.

Gas chromatography

The gas chromatograph used was a Shimadzu Model GC5AIE equipped with an ECD. The GC conditions were as follows: analytical column, 3 m \times 3 mm I.D., glass; column packing, 2% DEGS + 0.5% H₃PO₄ on Chromosorb W 80–100 mesh, acid-washed and silanized; detector ECD ⁶³Ni (10 mCi); carrier gas (nitrogen) flow-rate, 50 ml/min; column temperature, 170°; injection port temperature 200°; detector temperature, 250°, pulsed voltage (voltage, 48 V, pulsed width,

8 μ sec, mode, Hi). To determine the response factor (F_i)⁹⁻¹⁵ of the eight bromophenols investigated, the solution was prepared by dissolving the eight bromophenols (0.12–0.32 μ g), and *o*-dinitrobenzene (0.4 μ g) as an internal standard, in 1 ml of ethanol. 3 μ l of the sample solution was injected with a Hamilton microsyringe (10 μ l) into the column and detected with an ECD. The reproducibility and uniformity of the quantitative response to the ECD for the eight bromophenols were evaluated by the response factors (F_i):

$$F_i = \frac{A_s}{W_s} \cdot \frac{W_i}{A_i}$$

where A_s and W_s are the peak area and weight of the internal standard (*o*-dinitrobenzene), respectively, and A_i and W_i are the peak area and weight of the bromophenols.

RESULTS AND DISCUSSION

Fig. 1 shows a typical gas chromatogram of the eight bromophenols with an ECD. The bromophenols are eluted within about 35 min, and the bromophenols derived from phenol, *o*-, *m*- and *p*-cresol were well separated. The reproducibility and uniformity of the response of an ECD were good, as shown in Table I by the response factors (F_i) of the bromophenols.

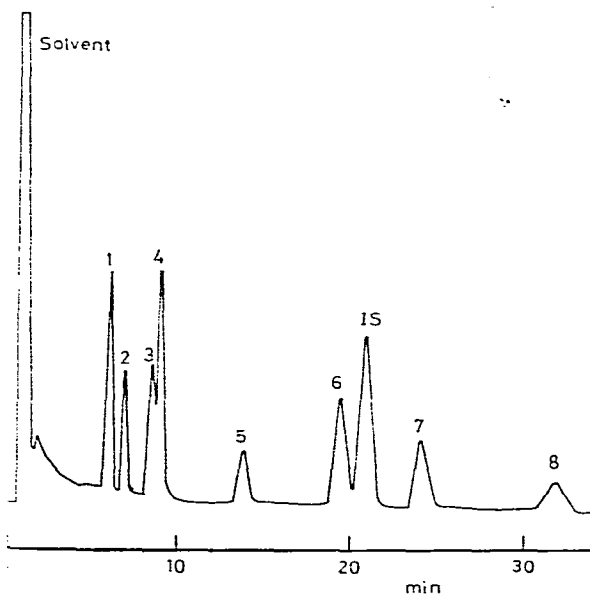


Fig. 1. Typical gas chromatogram of eight bromophenols with an ECD. Peak numbers: 1 = di-bromo-*o*-cresol (0.6 ng); 2 = tribromo-2,5-xyleneol (0.4 ng); 3 = bromo-2,3-xyleneol (0.6 ng); 4 = dibromo-*p*-cresol (0.9 ng); 5 = tribromo-3,4-xyleneol (0.6 ng); 6 = tribromophenol (0.5 ng); IS (internal standard) = *o*-dinitrobenzene (1 ng); 7 = tribromo-*m*-cresol (0.5 ng); 8 = tribromo-3,5-xyleneol (0.5 ng). GC conditions: analytical column, 3 m \times 3 mm I.D., glass; column packing, (2% DEGS + 0.5% H_3PO_4) on Chromosorb W, 80–100 mesh, acid-washed and silanized; detector, ^{63}Ni (10 mCi) ECD; carrier gas (nitrogen) flow-rate, 50 ml/min; column temperature, 170°; injection port temperature, 200°; detector temperature, 250°; pulsed voltage (voltage, 48 V; pulsed width, 8 μ sec); range, 8 (\times 0.01 V); sensitivity, 10^2 (\times M Ω).

TABLE I

RESPONSE FACTOR (F_1) VALUES AND STANDARD DEVIATION OF EIGHT BROMOPHENOLS

Parent phenol	F_1 value and standard deviation	Number of bromine atoms in derivative*
Phenol	0.68 \pm 0.01	3
<i>o</i> -Cresol	1.06 \pm 0.09	2
<i>m</i> -Cresol	0.87 \pm 0.03	3
<i>p</i> -Cresol	1.08 \pm 0.03	2
2,3-Xylenol	1.16 \pm 0.03	—
2,5-Xylenol	1.08 \pm 0.02	3
3,4-Xylenol	2.26 \pm 0.06	3
3,5-Xylenol	1.47 \pm 0.07	3

* Ref. 8.

Fig. 2 shows the calibration curves for three bromophenols. The detector response (peak area) of an ECD to each bromophenol was a straight line in the range 0.04–3 ng. The minimum detectable amount of these bromophenols was *ca.* 0.01 ng; therefore, the bromoderivatives have sufficient electron capturing properties. The sensitivity of the ECD to the bromophenols was *ca.* 100 times greater than that of a FID to the free phenols¹⁶.

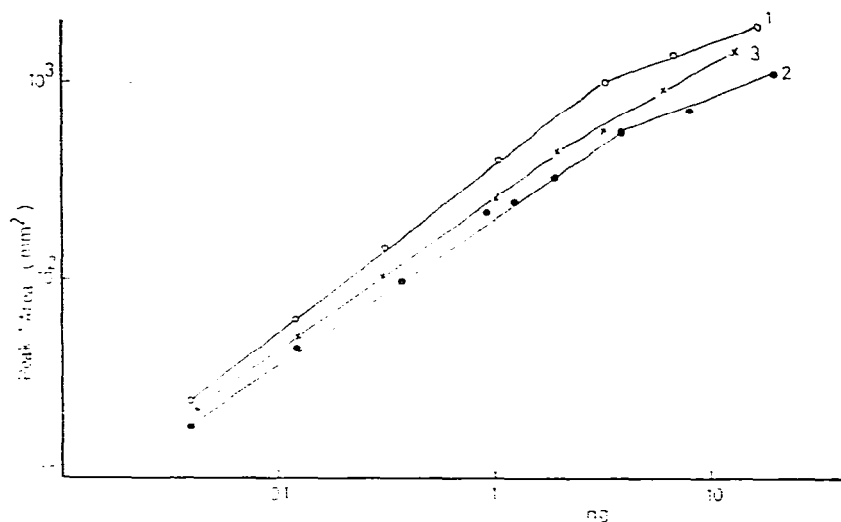


Fig. 2. Calibration curves for three bromophenols with an ECD. 1 = Tribromophenol; 2 = di-bromo-*o*-cresol; 3 = tribromo-*m*-cresol.

The interfering effects of other phenols in the present method were as follows: 2,4-xylenol, 2,6-xylenol, *o*-ethylphenol and *p*-ethylphenol at a ratio of 100:1 (the four phenols/the other eight phenols) had no effect on the detection of the 10^{-8} mol of the eight bromophenol derivatives in 1 ml of ethanol.

The effect of mol ratio (bromine/phenols) on reaction percentage is listed in Table II. The optimum mol ratio (bromine/phenols) was over 400 in the preparation of samples of 10^{-8} mol of each phenol in 1 ml of ethanol.

Fig. 3 shows the gas chromatograms of the bromophenols isolated from

TABLE II

EFFECT OF MOL RATIO (BROMINE/PHENOL) ON REACTION PERCENTAGE IN BROMOPHENOL DERIVATIVE FORMATION

Reaction conditions were: room temperature; time, 4 h; concentration of seven phenols, 10^{-8} mol/ml (ethanol).

Parent phenol	Reaction percentage at mol ratio of								
	1:1	2:1	4:1	10:1	20:1	40:1	100:1	200:1	400:1
Phenol	—	—	—	—	3	7	31	46	100
<i>o</i> -Cresol	—	2	8	49	95	100	100	100	100
<i>m</i> -Cresol	—	—	—	14	32	100	100	100	100
<i>p</i> -Cresol	—	5	11	84	100	100	100	100	100
2,5-Xylenol	2	2	7	96	100	100	100	100	100
3,4-Xylenol	—	4	8	100	100	100	100	100	100
3,5-Xylenol	—	—	3	35	100	100	100	100	100

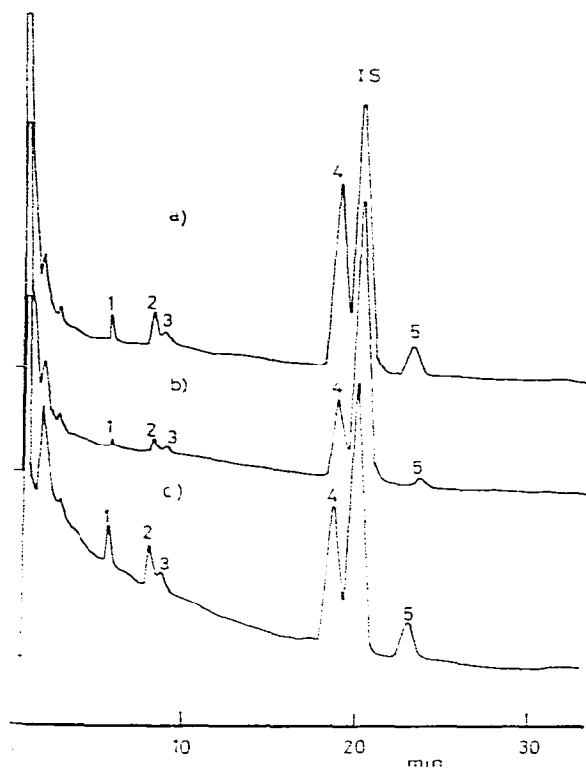


Fig. 3. Typical gas chromatograms of bromophenols, isolated from the exhaust gas of three different cars. GC conditions as in Fig. 1; $5 \mu\text{l}$ of sample solution injected. Peaks: 1 = dibromo-*o*-cresol; 2 = bromo-2,3-xylenol; 3 = dibromo-*p*-cresol; 4 = tribromophenol; IS (internal standard) = *o*-dinitrobenzene; 5 = tribromo-*m*-cresol. Amounts detected are listed in Table III.

TABLE III

AMOUNTS OF BROMIDES DETECTED IN THE EXHAUST GAS OF THREE DIFFERENT CARS

See Fig. 3 for identification of compounds.

Car	Amount of sample (l)	Detected amount of bromide (ng)					
		1	2	3	4	IS	5
a	25	0.2	0.3	0.12	3.7	5.0	0.35
b	12	0.1	0.16	0.1	1.0	5.0	0.06
c	25	0.2	0.4	0.15	2.5	5.0	0.5

three samples of car exhaust gas using the present method. The amounts detected are listed in Table III. The exhaust gases were produced by idling engines. The procedure for collecting the gases and preparing the bromophenols was as follows. The exhaust gases (12–25 l) were collected and condensed by the cold trap method with liquid oxygen and dissolved in 5 ml of ethanol. 10 ml of 0.1 *N* sodium hydroxide aqueous solution was added to the ethanol solution and heated with an IR lamp (100 V, 375 W, type R) for 0.5 h. The remaining solution was transferred to a 100-ml separator funnel, and 10 ml of *n*-hexane was added. After shaking for 1 min, the aqueous solution layer was separated, heated with the IR lamp for 10 min, cooled to room temperature, and transferred to another 100-ml separator funnel. A 1-ml volume of 6 *N* hydrochloric acid was added, followed immediately by 5 ml of diethyl ether. After shaking vigorously for 1 min, the ether layer was separated and concentrated to 0.5 ml by bubbling through nitrogen for 40 min at a flow-rate of 80 ml/min. Ethanol (1 ml) was added, after which the mixture again concentrated to 1 ml by bubbling through nitrogen for 15 min at a flow-rate of 80 ml/min. A 100- μ l volume of 1% (w/v) bromine-ethanol solution was added with a 500- μ l Hamilton microsyringe (750) to the concentrated ethanol solution, which was kept at room temperature for 4 h. A 10- μ l aliquot of the resulting solution was dissolved in 1 ml of ethanol, and 5 μ l of this sample solution was injected into column. As shown in Fig. 3, at least five bromophenols were identified and separated completely, and including a high concentration of phenol.

Table IV lists the quantitative results of the phenols detected in the three car sample gases. The detected concentrations of phenol agree closely several earlier reports^{17,18}. The recovery of the seven phenols using the overall treatment with a cold trap containing liquid oxygen and bromination was measured by using 10 μ l of the standard solution of phenols in ethanol (10^{-5} mol/10 ml). The results of this recovery test were 91–100% for each phenols.

TABLE IV

PHENOL AND CRESOLS DETECTED IN CAR EXHAUST GAS

Substance	Car a (μ g/25 l)	Car b (μ g/12 l)	Car c (μ g/25 l)
Phenol	0.84	0.48	0.57
<i>o</i> -Cresol	0.06	0.07	0.06
<i>m</i> -Cresol	0.09	0.03	0.12
<i>p</i> -Cresol	0.04	0.07	0.05

Barber *et al.*¹⁷ have reported the separation and identification of phenols in some car exhaust gases using a glass capillary column coated with tricresyl phosphate and H₃PO₄, with a chromatographic time of 20 min. However, the sample volume required is 3000–5000 l.

Seizinger *et al.*¹⁸ have reported the separation and identification of oxygenates in some exhaust gases, but the peaks of the cresols were not separated. The sample volumes in the present method were as small as 12–25 l, and furthermore the method is sensitive and rapid.

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