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SEPARATION OF ISOMERIC ALKYLPHENOLS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC AND GAS–LIQUID CHROMATOGRAPHIC TECHNIQUES

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SUMMARY

The separation of 13 isomeric alkylphenols has been studied by high-performance liquid (HPLC), gas–liquid (GLC) and high-performance thin-layer chromatographic (HPTLC) techniques. It has been shown that the separation of isomeric alkylphenols is dependent upon the adsorption, the polarity of the solvent systems and the configuration of the compounds when using HPLC technique. The separation of all the 13 isomeric alkylphenols by GLC is also possible but the long analysis times and instability of the stationary phase at higher temperatures makes it inferior to HPLC. It has also been shown that HPLC is more powerful than HPTLC. A modification of the injection port for increasing the life of HPLC septa is suggested.

INTRODUCTION

The analysis of isomeric alkylphenols is of interest because of their presence in tar acids[1] which may be used as the starting material for phenol–formaldehyde polycondensates as well as for analysis[2,3] of final resins by pyrolysis, giving phenols of composition related to that of the raw material used. This class of compounds has also been recognized[4] as a major source of pollutants. Phenols are introduced into the environment through the discharge of industrial wastes and the decomposition of pesticides and herbicides. Further, the principal simple phenols have been reported[5] to be present in human urine. These phenols may be derived not only from the dietary intake of proteins, fats, smoked foods such as meat and water, but also from a wide variety of exogenous materials. A rapid, simple and effective method of analysis for phenolic compounds would be useful.

The complete analysis of a mixture of phenol, the three methylphenols, the three ethylphenols and the six dimethylphenols, which are generally present in a tar acid cut (b.p. 180–226°), is very difficult on conventional packed columns[2] using polar or non-polar stationary phases. These compounds have a high polarity and low

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vapour pressure at moderate temperatures. In addition, certain pairs of these compounds have nearly the same vapour pressures, *e.g.*, 3-methylphenol and 4-methylphenol; 2,4-dimethylphenol and 2,5-dimethylphenol; 3-ethylphenol and 2,3-dimethylphenol; and 4-ethylphenol and 3,5-dimethylphenol.

A comprehensive review[2] of phenol analysis, covering the literature from 1956 to 1965, was published. The use of high-efficiency capillary columns[6–8] using specific selective stationary phases is necessary to obtain a good resolution of these compounds. This procedure leads to long analysis times because such columns are long and the best stationary phases are not stable at high temperatures. Graphitized carbon black[9] deposited as a thin layer on the walls of a glass capillary column has been shown[10,11] to be a very effective absorbent for the separation of phenolic isomers. A much better analysis[12,13] is obtained with derivatives rather than with the free phenols, but this technique has some limitations[14]. Sterically hindered phenols can react slowly and incompletely and hydrolysis should also be taken in account.

The direct thin-layer chromatographic (TLC) separation of isomeric alkylphenols using silica gel as adsorbent has not been very successful owing to the limited resolution power of the adsorbent. It is believed[15] that gas–liquid chromatography (GLC) is superior to most other methods employed for the separation of isomeric phenols. However, derivatives of some isomeric alkylphenols have been reported[16] to be separated on silica gel plates. Detection limits and the semi-quantitative determination of phenol, the three methylphenols and the six dimethylphenols on ready-made impregnated sheets of silica gel have been reported[17]. Recently, a method for the identification of phenolic substances by means of six one-dimensional TLC systems and four spray reagents has been recommended[18].

Literature on the separation of isomeric alkylphenols using high-performance liquid chromatography (HPLC) is scanty. Some alkylphenols, whenever available with other classes of compounds, have been reported to be separated by HPLC. A mixture containing five phenols (phenol and 4-methyl-, 2,6-dimethyl-, 2,4-dimethyl- and 3,4-dimethylphenol) has recently been separated on a Chromosorb G (5–10-μm) column using HPLC[19].

This paper describes what we believe to be the first separation of almost all of the isomeric alkylphenols by HPLC. A modification to the injection port has been employed in order to avoid frequent rupture of the septa. The separation of these alkylphenols using two stationary phases on packed columns by GLC is also reported.

EXPERIMENTAL

Alkylphenols were obtained from Fluka (Buchs, Switzerland) and were of technical grade. Compounds with purity less than 98% were recrystallized from light petroleum (b.p. 60–70°) before use. 2,4-Dimethylphenol was the only compound to be used without purification and its composition was *ca.* 90% 2,4-dimethylphenol + 5–7% 2,5-dimethylphenol + methylphenol. Cyclohexane and methylene dichloride, used as solvents, were pure and did not show any impurities in the 250–400-nm UV range.

HPLC

A DuPont Model 830 high-performance liquid chromatograph equipped with

a DuPont Model 835 UV detector was used. The elution was monitored at 254 nm. The pumps were capable of operating at pressures up to 4500 psi. The following three columns were used: (1) a stainless-steel column (1000 × 3 mm I.D.), dry packed in the laboratory using 30-μm LiChrosorb SI 60 (Merck, Darmstadt, G.F.R.), as stationary phase; (2) a stainless-steel column (300 × 3 mm I.D.), packed in the laboratory with 10-μm LiChrosorb SI 60 (Merck) using the balanced-density slurry method[20]; (3) a pre-packed column of Zorbax Sil (250 × 2.1 mm I.D.), particle size 5 μm, obtained from DuPont (Wilmington, Del., U.S.A.).

Solutions (0.05%) of the alkylphenols were prepared in cyclohexane and 1.0-μl samples were injected below the surface of the column packing with a 5-μl Hamilton syringe for HPLC. Whenever the pressure was more than 1000 psi, the stop–flow injection method was employed.

Septa made of perfluorelastomer supplied by DuPont were used. A life of 20 or more injections at room temperature for these septa has been claimed. It was impossible to use these septa above 40° as they frequently ruptured. In order to overcome this difficulty, a packing of PTFE was provided at the injection port so that a minimal area of the septum would come into contact with the solvent. An excellent improvement in the life of the septa was achieved with this modification. Fig. 1 illustrates this PTFE packing.

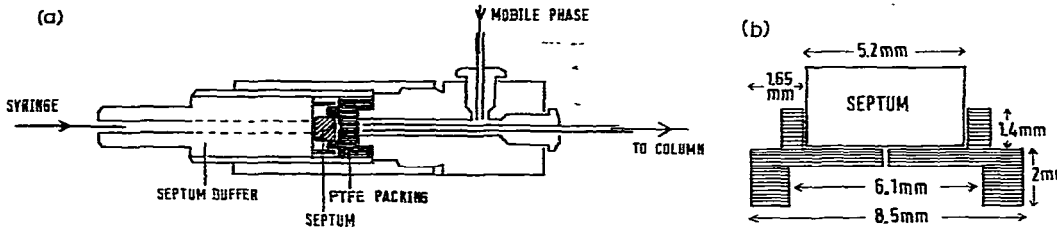

Fig. 1. (a) Schematic diagram of the injection port (Dimensions as in DuPont Accessories and Parts Catalogue) with PTFE packing. (b) Schematic diagrām of the PTFE packing and septum.

HPTLC

The pre-coated fluorescence silica gel high-performance thin-layer chromatographic (HPTLC) plates used in this investigation were obtained from Merck[21] and were dried at 105–110° for 30 min before use. The compounds were dissolved in methylene dichloride and nanogram amounts were applied with a glass capillary 10 mm from the edge of the plates. The plate was then placed inside the chamber for development. Pure cyclohexane, pure methylene dichloride and their mixtures in different proportions were used as solvent systems. The spots were observed under UV illumination.

GLC

A Varian Aerograph Model 1800 gas chromatograph equipped with a flame-ionization detector was used with nitrogen as the carrier gas.

Di-(3,3,5-trimethylcyclohexyl) phthalate (DTCHP), 2.5% on Chromosorb W, 80–100 mesh, and trimethylolpropane tripelargonate (Celanese ester No. 9), 15% on Celite 545, 60, 80–100 mesh, liquid phases were obtained from Perkin-Elmer

(Norwalk, Conn., U.S.A.) and were packed in glass columns (4 m × 3 mm I.D.) by the conventional method. These columns were conditioned before use as described in the instructions supplied by Perkin-Elmer. The other conditions were: oven temperature, see Table IV; injector temperature, 250°; and carrier gas (nitrogen) flow-rate, 25 ml/min.

RESULTS AND DISCUSSION

HPLC

The stainless-steel column (1000 × 3 mm I.D.) packed with LiChrosorb SI 60 of particle size 30 μm was first used for the HPLC separation of isomeric alkylphenols using different proportions of cyclohexane and methylene dichloride. Broad peaks were obtained for most of the phenols as their adsorption on this column was very strong. A mixture of 13 isomeric alkylphenols gave no more than four peaks. Known amounts of water were added to the methylene dichloride before mixing with cyclohexane in order to study the influence[21] of water on the separation of these alkylphenols, but no improvement in the separation was observed.

Table I gives the retention times of the 13 isomeric alkylphenols on LiChrosorb SI 60 (300 × 3 mm I.D.) of particle size 10 μm and on Zorbax Sil (250 × 2.1 mm I.D.) of particle size 5 μm using HPLC under different operating conditions. It can be seen that the performances of both columns are similar, provided that the ratio of cyclohexane to methylene dichloride is 1:1. The following compounds were not separated: 2-methyl-, 2,5-dimethyl- and 2,3-dimethylphenols from each other; 3-ethyl-

TABLE I

RETENTION TIMES OF ISOMERIC ALKYLPHENOLS BY HPLC USING SILICA GEL COLUMNS OF PARTICLE SIZE 30 AND 5 μm

(A) 300 × 3 mm I.D. column of LiChrosorb SI 60 (30 μm); pressure, 2500 psi; eluent, cyclohexane–methylene dichloride (1:1); flow-rate, 1.4 ml/min; temperature, 25°. (B) 250 × 2.1 mm I.D. column of Zorbax Sil (5 μm); pressure, 3300 psi; eluent, cyclohexane–methylene dichloride (1:1); flow-rate, 0.8 ml/min; temperature, 25°. (C) Column as B; pressure, 3800 psi; eluent, cyclohexane–methylene dichloride (2:1); flow-rate, 0.8 ml/min; temperature, 25°. (D) As C, except flow-rate, 0.95 ml/min; temperature, 40°.

Compound	*Retention time (min)*			
	A	*B*	*C*	*D*
2,6-Dimethylphenol	2.2	2.4	3.6	2.8
2-Ethylphenol	3.6	3.6	5.9	4.2
2-Methylphenol	3.8	4.5	7.4	5.3
2,5-Dimethylphenol	3.8	4.5	7.4	5.3
2,3-Dimethylphenol	4.0	4.5	7.4	5.3
2,4-Dimethylphenol	4.2	4.8	8.2	6.0
3-Ethylphenol	6.4	6.8	12.0	8.7
Phenol	6.4	6.8	12.0	8.7
4-Ethylphenol	7.0	7.6	13.4	9.6
3-Methylphenol	7.0	7.6	13.4	9.6
4-Methylphenol	7.0	7.6	14.0	10.2
3,5-Dimethylphenol	7.0	7.6	14.0	10.2
3,4-Dimethylphenol	—	—	14.8	11.0

TABLE II

RETENTION TIMES OF ISOMERIC ALKYLPHENOLS BY HPLC AT DIFFERENT TEMPERATURES

(E) 250 × 2.1 mm I.D. column of Zorbax Sil (5 μm); pressure 2200 psi; eluent, cyclohexane–methylene dichloride (3:1); flow-rate, 0.56 ml/min; temperature 46°. (F) As E, except pressure, 2000 psi; flow-rate, 0.54 ml/min; temperature 53°. (G) As E, except pressure, 2000 psi; flow-rate, 0.61 ml/min; temperature, 63°.

Compound	*Retention time (min)*		
	E	*F*	*G*
2,6-Dimethylphenol	5.7	5.0	3.6
2-Ethylphenol	9.2	8.0	5.2
2-Methylphenol	11.7	10.0	6.6
2,5-Dimethylphenol	11.7	10.0	6.6
2,3-Dimethylphenol	12.2	10.5	6.8
2,4-Dimethylphenol	13.3	11.5	7.3
3-Ethylphenol	18.9	15.8	10.0
Phenol	19.6	16.5	10.6
4-Ethylphenol	20.8	17.3	10.9
3-Methylphenol	20.8	17.3	10.9
4-Methylphenol	21.8	18.4	10.9
3,5-Dimethylphenol	21.8	18.4	10.9
3,4-Dimethylphenol	24.3	19.8	12.6

TABLE III

RETENTION TIMES OF ISOMERIC ALKYLPHENOLS BY HPLC USING DIFFERENT PROPORTIONS OF THE COMPONENTS OF THE SOLVENT SYSTEM

(H) 250 × 2.1 mm I.D. column of Zorbax Sil (5 μm); pressure 2200 psi; eluent, cyclohexane–methylene dichloride (4:1); flow-rate, 0.53 ml/min; temperature, 42°. (I) Column as H; pressure, 2000 psi; eluent, cyclohexane–methylene dichloride (4:1); flow-rate, 0.5 ml/min; temperature, 50°. (J) Column as H; pressure, 2100 psi; eluent, cyclohexane–methylene dichloride (6:1); flow-rate, 0.5 ml/min; temperature, 48°. (K) Column as H; pressure, 2500 psi; eluent, cyclohexane–methylene dichloride (7.5:1); flow-rate, 0.6 ml/min; temperature, 48°.

Compound	*Retention time (min)*				*Peak No. (see Fig. 2)*
	H	*I*	*J*	*K*	
2,6-Dimethylphenol	6.4	6.4	8.6	8.8	3
2-Ethylphenol	10.8	10.2	13.9	14.3	6
2-Methylphenol	14.2	12.8	17.8	18.2	2
2,5-Dimethylphenol	14.2	12.8	18.1	19.0	8
2,3-Dimethylphenol	14.9	13.3	18.9	20.7	9
2,4-Dimethylphenol	16.0	15.8	20.3	21.4	7
3-Ethylphenol	23.5	20.8	29.2	30.4	11
Phenol	24.2	21.3	32.0	33.8	1
4-Ethylphenol	25.7	22.8	33.5	35.1	10
3-Methylphenol	25.7	22.8	33.5	35.1	5
4-Methylphenol	27.1	23.8	34.2	37.0	4
3,5-Dimethylphenol	27.1	23.8	34.2	37.0	12
3,4-Dimethylphenol	30.0	26.4	37.6	40.6	13

phenol from phenol; and 4-ethyl-, 3-methyl-, 4-methyl- and 3,5-dimethylphenol from each other.

When the cyclohexane to methylene dichloride ratio was changed to 2:1, an extra peak was obtained (Table I, C) and 4-ethyl- and 3-methylphenol were separated from 4-methyl- and 3,5-dimethylphenol.

Table II gives the retention times of these alkylphenols at different temperatures using a cyclohexane to methylene dichloride ratio of 3:1. It can be seen that with an increase in the non-polar content in the solvent system, it was possible to separate 2,5-dimethyl- from 2,3-dimethylphenol and 2-ethyl- from phenol at 46° and 53°. Poor separations were found at 63° (Table II, G).

Table III gives the retention times of these isomeric alkylphenols using different proportions of the components of the solvent system. By further increasing the cyclohexane content, it was possible to separate 2-methylphenol from 2,5-dimethylphenol.

A typical separation of 11 isomeric phenols from a mixture containing 13 compounds is shown in Fig. 2. The separation of 4-ethyl- from 3-methylphenol and 4-methyl- from 3,5-dimethylphenol could not be achieved.

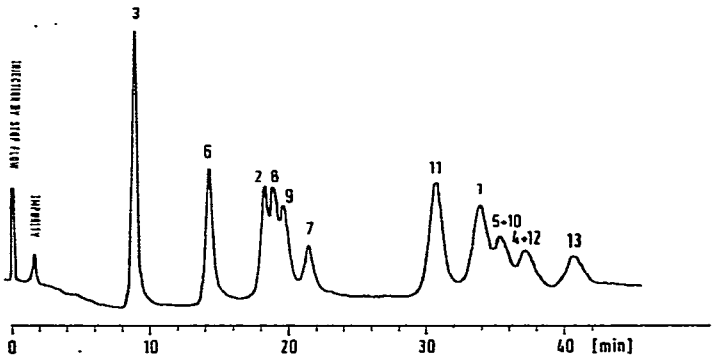

Fig. 2. Separation of isomeric alkylphenols by HPLC. For conditions and identification of peaks, see Table III, K.

It was found that the separation of these isomeric alkylphenols depends upon the adsorption phenomenon, the polarity of the solvent system and the configuration of the compounds. The resolving power of the solvent system could be increased by decreasing its polarity. *Ortho*-substituted compounds were eluted earlier than the corresponding *meta*-isomers, which is a result of the *ortho*-effect[22].

HPTLC

The compounds studied did not move from the starting point when pure cyclohexane was used as the solvent in HPTLC. By increasing the polarity of the solvent systems by addition of methylene dichloride, it was possible to move these compounds from the starting line. When pure methylene dichloride was used as the solvent in HPTLC, the maximum R_F value (0.39) was obtained for 2,6-dimethylphenol. However, the overall resolution of these phenols was very poor. When a mixture containing 13 compounds was spotted on an HPTLC plate using methylene dichloride as the solvent, only three spots were obtained. The ranges of the R_F values

TABLE IV

BOILING POINTS AND RELATIVE RETENTION TIMES OF ISOMERIC ALKYLPHENOLS BY GLC

Peak No. (see Fig. 3)	*Compound*	*Boiling point (°C)*	*DTCHP (2.5%)*			*Celanese ester No. 9 (15%) at 175°*
			155°	*135°*	*125°*	
1	Phenol	181.75	1 (16.9 min)	1 (38.3 min)	1 (47 min)	1 (28.5 min)
2	2-Methylphenol	190.95	1.30	1.34	1.34	1.25
3	2,6-Dimethylphenol	201.00	1.54	1.43	1.42	1.34
4	4-Methylphenol	202.30	1.56	1.67	1.72	1.55
5	3-Methylphenol	202.60	1.89	1.8	1.85	1.55
6	2-Ethylphenol	207.00	2.14	2.15	2.23	1.80
7	2,4-Dimethylphenol	210.00	2.18	2.27	2.36	1.94
8	2,5-Dimethylphenol	210.00	2.19	2.41	2.51	1.94
9	2,3-Dimethylphenol	218.00	2.59	2.85	2.98	2.35
10	4-Ethylphenol	218.20	2.85	2.93	3.11	2.35
11	3-Ethylphenol	219.00	2.88	3.15	3.34	2.45
12	3,5-Dimethylphenol	219.50	2.91	3.27	3.45	2.45
13	3,4-Dimethylphenol	225.00	3.10	3.59	3.81	2.81

of these three groups are as follows: phenol, 3-methyl-, 4-methyl-, 3,4-dimethyl-, 3,5-dimethyl-, 3-ethyl- and 4-ethylphenol, 0.16–0.17; 2-methyl-, 2,5-dimethyl-, 2,3-dimethyl-, 2,4-dimethyl and 2-ethylphenol, 0.24–0.27; 2,6-dimethylphenol, 0.39.

From these results, it appears that the resolving power of HPLC is superior to that of HPTLC for the compounds and conditions studied in this work.

GLC

Relative retention times of the 13 isomeric alkylphenols on two liquid phases using different temperatures are listed in Table IV. The elution sequence of these phenols follows the order of their boiling points. It was possible to separate all 13 alkylphenols using DTCHP as the stationary phase (Fig. 3), whereas only nine compounds were separated when Celanese ester No. 9 was used (Table IV). By employing

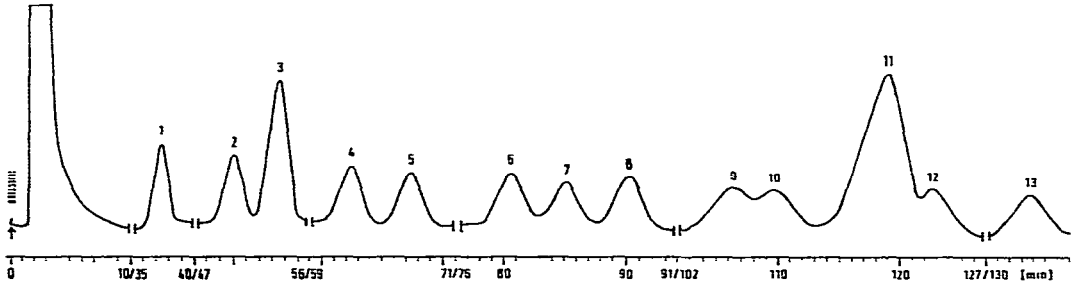

Fig. 3. Separation of isomeric alkylphenols on a glass packed column using DTCHP as stationary phase at 125°. For other conditions, see Experimental; for identification of peaks, see Table IV.

temperatures higher than 125°, the separation was found to be better on DTCHP. Unfortunately, DTCHP is unstable at higher temperatures.

CONCLUSIONS

This study established the chromatographic conditions for separating 13 isomeric alkylphenols by HPLC and GLC. These compounds were separated by HPLC on silica gels using cyclohexane and methylene dichloride as solvents. The adsorbent surface area and mobile phase polarity were varied so as to optimize the separations.

The complete GLC separation of a mixture of isomeric alkylphenols on a packed column using DTCHP as the stationary phase could be achieved. However, the long analysis times and the instability of the stationary phase at temperatures higher than 125° make this method inferior to HPLC.

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