

## THE ANALYSIS OF MIXTURES OF ISOMERIC BENZENOID COMPOUNDS BY GAS-LIQUID CHROMATOGRAPHY

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There have been several reports of the resolution of isomeric disubstituted benzenes by gas-liquid chromatography, some of which concern the development of new, and often specific, liquid phases. For example, the xylenes have been resolved by DESTY, GOLDUP AND SWANTON using 7,8-benzoquinoline as the liquid phase<sup>1</sup>, and by LANGER, ZAHN AND PANTAZOPOLOS using tetrahalophthalate esters<sup>2</sup>; the nitrotoluenes have been resolved by NORMAN using 2,4,7-trinitrofluorenone<sup>3</sup>; the chloronitrobenzenes by BOMBAUGH using Carbowax 1000<sup>4</sup>; and the cresols by PAYN using di-*n*-octyl sebacate<sup>5</sup>. There has not, however, been a systematic survey of the application of gas-liquid chromatography to the analysis of mixtures of isomeric disubstituted benzenes. The present work was designed to provide information about the suitability of a number of materials as liquid phases for the resolution of such mixtures, so as to facilitate selection of the best liquid phase for the quantitative analysis of any given mixture of benzenoid derivatives.

### EXPERIMENTAL

The apparatus was assembled in this laboratory. The column was a coiled copper tube of length 200 cm (and in one case 400 cm; see Table I) and diameter 4.5 mm. Nitrogen was used as the carrier gas, at an inlet pressure of about 70 cm, with atmospheric pressure at the outlet, the inlet pressure being adjusted to keep the flow at about 25 ml/min. A hydrogen-inject flame ionisation detector coupled to a Sunvic or Honeywell Brown recorder gave a linear response.

The columns were prepared as follows. A solution of the liquid phase in ether or chloroform was added to the solid support. The solvent was evaporated slowly on a steam bath, with stirring, and the material was dried at 110° for 1 h. It was packed into the column with the aid of an electric vibrator, and the copper tube was then coiled. The column was dried for at least 1 h at 120° in a flow of carrier gas before being used.

The following liquid phases were investigated: Apiezon L, dinonyl phthalate (DNP), polyethylene glycol + stearic acid (PEG), tritolyl phosphate (TTP), 2,4,7-

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trinitrofluorenone (TNF) and silicone gum rubber (SGR). A summary of the columns studied is given in Table I.

The benzenoid compounds were introduced into the column in 10% ethereal solution. 10  $\mu$ l samples were employed, and injection was by an Agla microsyringe. Compounds were examined both individually, to determine their retention times, and in mixtures with their isomers, to find if resolution was adequate for quantitative

TABLE I  
GAS CHROMATOGRAPHIC COLUMNS

Liquid phase	Solid support	% of liquid on solid (w/w)	Length (cm)	Max. temp. of operation ( $^{\circ}$ C)
Apiezon L	Firebrick (50-90 mesh)	20	200	200
DNP	Embacel	20	200	120
PEG	Embacel	5*	200	120
TTP	Embacel	20	200	150
TNF	Embacel	10	400	200
SGR	Glass microbeads (200 $\mu$ diameter)	1	200	150

\* Together with 2% stearic acid.

analysis of the mixtures. From the results, the relative peak separation,  $\alpha$ , and the fractional band impurity,  $\eta$ , were calculated, the latter by the method of GLUECKAUF<sup>6</sup>. Retention times, and values of  $\alpha$ , and  $\eta$ , for all the columns studied save that packed with SGR on glass microbeads (which was applicable to the resolution only of a small number of very high-boiling compounds; see below) are set out in Tables II and III. Only values of  $\eta$  less than 0.1 are quoted; for values greater than this, resolution was not satisfactory for quantitative analysis of the components of the mixtures.

#### DISCUSSION

A limiting factor in the use of DNP, PEG and TTP as liquid phases is their volatility at high temperatures, their maximum operating temperatures being 120 $^{\circ}$ , 120 $^{\circ}$  and 150 $^{\circ}$  respectively. For this reason, the higher boiling benzene derivatives listed in Tables II and III (such as the nitroanisoles) could not be studied with these materials as liquid phases since the compounds were not eluted at the maximum operating temperatures of the columns.

Apiezon L and TNF have maximum operating temperatures of about 200 $^{\circ}$ , and could be used to study all the compounds in Tables II and III. However, compounds of boiling-point above about 280 $^{\circ}$  were not eluted from columns packed with these materials. For some of these high-boiling compounds, SGR coated on glass microbeads (according to the description given by HISHTA *et al.*<sup>7</sup>) was satisfactory. The results obtained using this column are shown in Table IV.

The low proportion of the liquid phase, together with the very weak adsorptive

RETENTION TIMES (min) OF BENZENOID COMPOUNDS

Compound	Liquid phases and operating temp.					
	Apiezon L	Dimonyl phthalate	Polyethylene glycol	Trityl phosphate		
Fluorobromobenzenes	o-	90°	85°	90°	100°	100°
	m-	20.5	30	27	28	26
	p-	18.5	22	18.5	19.5	22
Dichlorobenzenes	o-	120°	120°	100°	120°	120°
	m-	66	42	125	76	26
	p-	59	38	87	53	20
Bromochlorobenzenes	o-	120°	120°	90°	100°	100°
	m-	30	26	88	70	68
	p-	30	26	96	70	68
Chlorotoluenes	o-	100°	120°	100°	120°	100°
	m-	10.5	20	33	17	27
	p-	10.5	9.2	18.5	17	18
Bromotoluenes	o-	100°	120°	100°	120°	100°
	m-	33	18	57	46.5	50
	p-	33	18.5	62	49.5	28
Tolunitriles	o-	100°	100°	120°	100°	120°
	m-	27.5	57	19	124	44
	p-	46	82	25.5	191	60
Nitrotoluenes	o-	150°	165°	120°	120°	145°
	m-	85	43	95	111	52
	p-	128	65	141	141	76
Fluoronitrobenzenes	o-	105°	135°	120°	120°	120°
	m-	129	47	86.5	very	83
	p-	62	38.5	70	flat	40

ANALYSIS OF ISOMERIC BENZENOID COMPOUNDS

Compound	105°	135°	120°	120°	120°	120°	140°
Fluoronitrobenzenes							
o-	129	47	41.5	86.5	120°	120°	140°
m-	62	38.5	25	70			51
p-	95	40.5	31.5	74.5			31
							36
Chloronitrobenzenes							
o-	150°	170°	120°	120°	145°	150°	150°
m-	18	10.5	104	not eluted	79	71	72
p-			85		59	53	55.5
			95.5		68	62	60
Bromonitrobenzenes							
o-	150°	120°	120°	120°	150°	170°	170°
m-	35.5	not eluted	not eluted	not eluted	very flat peaks after 4 h	122	68
p-	40					94	55.5
	42.5					101.5	59.5
Nitroanisoles							
o-	170°	120°	120°	120°	150°	170°	180°
m-	15	not eluted	not eluted	not eluted	not eluted	93	61.5
p-	15					66.5	46
	20					118	77.5
Chloroanisoles							
o-	120°	120°	120°	120°	130°	120°	120°
m-	21	29	26	59	48	63	63
p-		27	48	48	34	59	59
					39	59	
Bromoanisoles							
o-	120°	120°	120°	120°	140°	140°	140°
m-	38	44	95	95	91.5	59	59
p-			78	78	76.5	52	52
			82	82	80	59	59
Methyl tolyl ethers							
o-	120°	100°	80°	80°	80°	100°	120°
p-	24	27.5	75	75	75	31	20.5
	30	32	91	91	87	36.5	29
Chlorobenzyl chlorides							
o-	120°	120°	120°	120°	120°	120°	130°
m-	12.5	40.5	47	47	40	27	18
p-	14	50	59	59	47	33.5	22
	14	50	59	59	47	33.5	22

TABLE III  
COLUMN EFFICIENCIES<sup>a</sup>

Compound	Liquid phases and operating temp.																			
	Acrylam L.		Dimethyl phthalate		Polyethylene glycol		Trityl phosphate		Trinitrofluorenone											
	$\alpha$	$\eta$	$\alpha$	$\eta$	$\alpha$	$\eta$	$\alpha$	$\eta$	$\alpha$	$\eta$	$\alpha$	$\eta$								
Fluorobromobenzenes m- p- o- }	90°	1.00	65°	4 · 10 <sup>-2</sup>	1.13	90°	1.19	4 · 10 <sup>-2</sup>	1.18	100°	1.00	100°								
													1.11	1.22	10 <sup>-3</sup>	1.23	10 <sup>-2</sup>	1.22	10 <sup>-2</sup>	1.18
Dichlorobenzenes m- p- o- }	120°	1.00	120°	4 · 10 <sup>-3</sup>	1.23	100°	1.12	10 <sup>-1</sup>	1.12	100°	1.00	120°								
													1.12	1.28	5 · 10 <sup>-3</sup>	1.28	10 <sup>-5</sup>	1.30	4 · 10 <sup>-2</sup>	
Bromochlorobenzenes m- p- o- }	120°	Not resolved	120°	Not resolved	1.09	90°	1.08	100°	1.08	100°	Not resolved	100° and 120°								

Fluoronitrobenzenes	$\left. \begin{array}{l} m- \\ p- \\ o- \end{array} \right\}$	105°		120°		120°		120°		120°	
		1.53	10 <sup>-12</sup>	1.26	10 <sup>-3</sup>	1.06		Not resolved		1.28	2 · 10 <sup>-2</sup>
		1.36	10 <sup>-8</sup>	1.32	3 · 10 <sup>-4</sup>	1.16	10 <sup>-3</sup>			1.63	10 <sup>-4</sup>
Chloronitrobenzenes	$\left. \begin{array}{l} m- \\ p- \\ o- \end{array} \right\}$	150 and 170°		120°		150°		150°		150°	
		Not resolved		1.12	2 · 10 <sup>-2</sup>	1.17	2 · 10 <sup>-2</sup>	1.08	10 <sup>-1</sup>		
				1.09	5 · 10 <sup>-2</sup>	1.14	4 · 10 <sup>-2</sup>	1.20	10 <sup>-3</sup>		
Bromonitrobenzenes	$\left. \begin{array}{l} o- \\ m- \\ p- \end{array} \right\}$	150°				150°		150°		150°	
		1.13	10 <sup>-2</sup>			Not resolved		$\left. \begin{array}{l} m- \\ p- \\ o- \end{array} \right\}$	1.20	5 · 10 <sup>-3</sup>	
		1.06	10 <sup>-1</sup>					1.08	10 <sup>-1</sup>		
Nitroanisoles	$\left. \begin{array}{l} m- \\ o- \\ p- \end{array} \right\}$	170°								170°	
		1.00								1.40	5 · 10 <sup>-10</sup>
		1.33	10 <sup>-4</sup>							1.27	10 <sup>-5</sup>
Chloroanisoles	$\left. \begin{array}{l} m- \\ p- \\ o- \end{array} \right\}$	120°		120°		120°		130°		120°	
		Not resolved		Not resolved		1.00		1.15		1.00	
						1.23	10 <sup>-2</sup>	1.23	10 <sup>-3</sup>	1.07	
Bromoanisoles	$\left. \begin{array}{l} m- \\ p- \\ o- \end{array} \right\}$	120°		120°		120°		140°		140°	
		Not resolved		Not resolved		1.05		1.05		1.13	
						1.16	10 <sup>-1</sup>	1.14	10 <sup>-1</sup>	1.00	
Methyl tolyl ethers	$\left. \begin{array}{l} o- \\ p- \end{array} \right\}$	120°		80°		80°		80°		100°	
		1.25	5 · 10 <sup>-2</sup>	1.17	3 · 10 <sup>-2</sup>	1.21	10 <sup>-2</sup>	1.16	2 · 10 <sup>-2</sup>	1.47	10 <sup>-2</sup>
Chlorobenzyl chlorides	$\left. \begin{array}{l} o- \\ m- \\ p- \end{array} \right\}$	120°		120°		120°		120°		120°	
		1.22	10 <sup>-2</sup>	1.23	10 <sup>-2</sup>	1.26	10 <sup>-2</sup>	1.18	10 <sup>-2</sup>	1.24	10 <sup>-2</sup>
		1.00		1.00		1.00		1.00		1.00	

\* Where a set of isomers was examined on a liquid phase at two temperatures, values of  $\alpha$  (and  $\eta$ ) are recorded only for the temperature at which resolution was more satisfactory.

TABLE IV  
CHROMATOGRAPHIC DATA FOR SGR-GLASS MICROBEADS COLUMN

Compounds	Column temp. °C	Inlet heater temp. °C	Number of theoretical plates	Retention time (min)	
Nitrobenzyl chlorides	80	230	200	2-	13
				3-	17.5
				4-	17.5
Dinitrobenzenes	100	250	90	<i>m</i> -	9.5
				<i>p</i> -	10.5
Chlorobiphenyls	110	260	300	2-	26
				3-	35
				4-	39
Nitrobiphenyls	150	275	150	2-	7.5
				3-	14
				4-	14.5

properties of the glass beads, enabled materials of boiling-point more than 200° above the temperature of the column to be eluted with short retention times. The efficiency of the column, however, was low: the number of theoretical plates, calculated by the method recommended by JOHNSON AND STROSS<sup>8</sup>, was never more than 300, and in no case were *meta*- and *para*-isomers resolved. Despite this low efficiency, resolution was adequate for estimation of the proportion of *ortho*-isomer in mixtures of the nitrobenzyl chlorides, chlorobiphenyls and nitrobiphenyls.

For the quantitative analysis of the mixtures examined, it was necessary that the value of the fractional band impurity, with reference to each pair of components in the mixture, be less than 0.1. When this condition was fulfilled, the peak area produced by each isomer in a mixture (measured by constructing triangles made up of tangents to the Gaussian curves and the intercepts on the base line) was proportional to the weight of isomer in the mixture to within  $\pm 2\%$ . Thus, such mixtures could be rapidly and accurately analysed by using the appropriate liquid phase. Applications of such analytical procedures have recently been described by NORMAN and his coworkers<sup>9</sup>. The *ortho*-*meta*-*para*-ratios of the following groups of isomeric compounds can be determined to within  $\pm 2\%$  in this way, using the liquid phases and temperatures shown:

Fluorobromobenzenes	DNP, 68°; PEG, 90°; TTP, 100°
Dichlorobenzenes	TTP, 100°
Tolunitriles	TTP, 100°; Apiezon L, 100°
Nitrotoluenes	Apiezon L, 150°; DNP, 120°; TNF, 145°
Fluoronitrobenzenes	Apiezon L, 150°; DNP, 120°; TNF, 120°
Chloronitrobenzenes	DNP, 120°; TNF, 150°
Bromonitrobenzenes	TNF, 150°
Nitroanisoles	TNF, 170°
Chloroanisoles	TTP, 130°

It is difficult to discern a pattern in the above results from which conclusions may be drawn concerning the mode of action of the liquid phases, but certain general observations may be made.

(i) Apiezon L was the least selective of the liquids studied. Only the tolunitriles, itrotoluenes and fluoronitrobenzenes were satisfactorily resolved by this liquid phase, and these groups of isomers were resolved at least as well by other liquids. The less polar groups of isomers were not well resolved on Apiezon L. Of the liquid phases examined, Apiezon L was the only non-polar one; polar liquids may be more suitable for resolution of the polar aromatic compounds studied here, acting, in a differential manner, by dipole-dipole interactions.

(ii) DNP, which is a moderately polar liquid, is fairly selective towards isomeric benzenoid compounds when isomers differ in dipole moment (*e.g.*, nitrotoluenes, fluoronitrobenzenes, chloronitrobenzenes). The more strongly polar liquid, PEG, shows a higher selectivity than DNP towards these same groups of isomers (see Table III). Further support for the view that the absorption process for these liquid phases involves a dipolar interaction is derived from the fact that PEG, at the same concentration and temperature as DNP, retains benzenoid compounds much more strongly: in each case the retention times were longer for the former column, while the chloronitrobenzenes were not eluted from PEG (see Table II).

(iii) TTP was the most satisfactory liquid phase for the following groups of isomers: dichlorobenzenes, bromochlorobenzenes, chloroanisoles and bromoanisoles. Compounds containing a halogen or ether function were not satisfactorily resolved on any other liquid except when the second substituent was a strongly polar one, such as fluoro or nitro. On the other hand, TTP was the least satisfactory liquid phase for compounds containing nitro groups.

(iv) TNF was the best liquid phase for nitro compounds. For example, it gave better resolution of the fluoronitrobenzenes, bromonitrobenzenes and nitroanisoles than the other liquids. (The nitroanisoles were either not eluted or not resolved by the other columns.) Since the nitro compounds were the most highly polar of the aromatic compounds studied, it appears that TNF acts by holding the nitro compounds so that the electron-deficient benzenoid ring is close to (one of) the electron-rich nitro-groups of TNF, while the nitro-group of the benzenoid compound is close to the fluorenone nucleus. Such a dipolar interaction is stereochemically reasonable.

There seems no evidence that TNF operates by forming charge-transfer complexes with the benzenoid compounds, for in that case such strong differentiation between isomeric nitro compounds would not be expected, since the electron-deficient benzenoid nuclei in these compounds should be ineffective donors in charge-transfer complexing.

#### SUMMARY

Retention data are recorded for nineteen groups of isomeric disubstituted benzenes on the stationary phases Apiezon L, dinonyl phthalate, polyethylene glycol-stearic acid, tritolyl phosphate, 2,4,7-trinitrofluorenone and silicone gum rubber. The conditions for the accurate determination of the *ortho*-:*meta*-:*para*-ratios of many of these mixtures are reported, and the particular applications of each of the liquid phases examined are discussed.



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