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GAS–LIQUID CHROMATOGRAPHIC RETENTION INDICES OF 296 NON-DRUG SUBSTANCES ON SE-30 OR OV-1 LIKELY TO BE ENCOUNTERED IN TOXICOLOGICAL ANALYSES

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1. INTRODUCTION

Recent advances in gas–liquid chromatography (GLC) have resulted in the widespread availability and use of highly selective detectors which enable toxicologists to detect smaller quantities of drug substances in their extracts than has previously been possible. The use of selective detectors such as the electron capture detector (ECD) for the identification of amenable compounds and the heated alkaline bead thermionic phosphorus/nitrogen detector (PND) for the detection of phosphorus and nitrogen containing compounds was anticipated to remove many of the extraneous peaks frequently observed when non-selective GLC detectors such as the flame ionization detector (FID) were used. The use of these selective detectors has indeed increased the sensitivity of detection of many drug classes, but has exchanged one set of problems for another. Hitherto undetected non-drug substances which interfere in many toxicological analyses are now observed.

One of the major groups of these interfering compounds encountered during

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toxicological analyses is the plasticizers. The term plasticizer as used here encompasses a wide range of compounds, usually esters of alcohols and dibasic or tribasic acids, which are also used in lubricants, coatings, propellants and PVC products etc.[1]. These substances have been introduced into biological samples prior to toxicological analyses from containers in which specimens were collected or stored[2–5] and have also been observed to compete with drugs for protein binding sites[6,7].

Plasticizers exhibit retention characteristics similar to those of drugs[8], but are by no means the only interfering compounds found during the GLC of toxicological extracts. Other compounds from plastics and rubber, *e. g.* antioxidants and plastic additives, as well as compounds naturally occurring in biological materials or which are formed as part of a putrefactive process may interfere with an analysis. Pesticides, food additives, flavours, fragrances and some scintillation chemicals are also observed from time to time. We have therefore examined the chromatographic characteristics of these interfering compounds, as well as compounds used as internal standards to allow their quick separation and identification.

The low polarity stationary phases SE-30 or OV-1 have been shown to be the most suitable for toxicological analyses[9] and are probably the most widely used stationary phases for the routine gas chromatography of drug substances[10–15]. Therefore a compilation of retention indices for 296 of these non-drug substances likely to be present in extracts of body fluids and tissues has been made using SE-30 or OV-1 as the stationary phase to update the compilation of data for 480 drugs made by Moffat[16].

2. EXPERIMENTAL

GLC of compounds on OV-1 was performed using a 4 m × 3 mm I.D. glass column in a Perkin-Elmer F17 gas chromatograph fitted with FID and PND. Helium carrier gas, maintained at 50 ml/min, was split 50:50 after leaving the column enabling chromatograms to be recorded showing the responses from two detectors simultaneously. OV-1 stationary phase (3%, w/w) was coated on Chromosorb W HP (80–100 mesh). Simultaneous detection was achieved using twin amplifiers and a dual pen recorder.

GLC of compounds on SE-30 was performed using a 1.5 m × 4 mm I.D. glass column packed with 3% (w/w) SE-30 on Chromosorb G HP (80–100 mesh) in a Pye 104 gas chromatograph fitted with ECD and FID. Nitrogen carrier gas was maintained at 60 ml/min and the column effluent split 50:50 as above to each detector.

Even-numbered straight-chain hydrocarbons were used as references for the calculation of retention indices[17]. Retention times were measured from the solvent front using either an integrator or a ruler.

A magnetic card programmable calculator was used to perform a least-squares regression analysis of log retention time against retention index (carbon number × 100). A correlation coefficient of 0.999 was easily obtained. This method was most convenient but readily acceptable results could be obtained using semi-log graph paper. Data was obtained for compounds with retention indices between 695 and 3800. Compounds were eluted by adjusting the oven temperatures so that the retention times of compounds were between 2 and 20 min.

Published data was used to supplement this compilation with values for pesticides. This information, published as relative retention times on DC-200[18] was transformed into retention indices on SE-30 after calibration curves of log relative retention times on DC-200 plotted against retention indices on SE-30 had been constructed for those compounds for which both sets of data were available.

Mass spectral characterisation of some plasticizers exhibiting multiple peaks by GLC were measured using a VG Micromass 16F mass spectrometer linked with a Pye series 104 gas chromatograph.

3. RESULTS AND DISCUSSION

3.1. Compilation of data

The compounds examined during this work have been classified into seven general categories (Table I) and the abbreviations corresponding to each category have been inserted into the subsequent tables at the appropriate places. Table 2 comprises a list of retention indices for compounds arranged in alphabetical order and the same data, with exception of those giving multiple peaks, are rearranged into ascending order of retention indices in Table 3. The names of the compounds used in this study were those in the *Merck Index*[19] or for the economic poisons the *Nanogen Index*[20].

Most of the data in Tables 2 and 3 were generated on the 4-m 3% OV-1 column. Thompson *et al.*[18] published data for 52 pesticides on the dimethylsilicone

TABLE 1

TABLE OF ABBREVIATIONS USED IN THE TABLES OF RETENTION DATA

Classification	
A	Antioxidants
D	Putrefactive and endogenous compounds
E	Pesticides (economic poisons)
F	Food additives, flavours and fragrances
I	Internal standards
P	Plasticisers, plastic additives, vulcanising agents
S	Scintillation reagents
Compound abbreviation	
BBO	2,5-Di-(4-biphenyloxazole)
BBOT	2,5-Bis(5′-*tert.*-butylbenzoxazoyl(2′))thiophene
BHC	Benzene hexachloride
CDEC	2-Chloroallyldiethyldithiocarbamic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
DCPA	Dimethyl-2,3,5,6-tetrachloroterephthalate
DDA	2,2-Bis(4-chlorophenyl)acetic acid
DDE	1,1-Dichloro-2,2-bis(4-chlorophenyl)ethylene
DDT	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane
PBD	2-Phenyl-5-(4-biphenylyl)-1,3,4-oxadiazole
POPOP	1,4-Bis(5-phenyloxazolyl-2)benzene
PPO	2,5-Diphenyloxazole
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
TDE *o,p′*	1,1-Dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
TDE *p,p′*	1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane

TABLE 2

RETENTION INDICES OF 296 NON-DRUG COMPOUNDS, USING SE-30 OR OV-1 AS THE STATIONARY PHASE, ARRANGED IN ALPHABETICAL ORDER OF COMPOUND NAME

NO = No peak observed; MP = multiple peaks, *i.e.* more than three.

Compound	*Retention index*	*Classification*
Acetylcholine	NO	D
Acetyltributyl citrate	2253	P
Acetyltriethyl citrate	1730	P
Adenine	NO	D
Aldrin	1950*	E
Alphanol 610*** (esters of C6–10 linear alcohols)	MP	P
3-Amino-2-dimethylbenzene	1178	I
5-Aminoquinoline	1598	I
Anethole	1284	F
Anilazine	2010	E
Anthracene	1711	I
9,10-Anthracenedicarbonitrile	2288	I
Atrazine	1705**	E
Azinphosmethyl	2430**	E
Azobenzene	1556	I
Barkite B***	MP	P
BBO	3710	S
BBOT	2745	S
	3750§	
Benzophenone	1611	F
Benzyl alcohol	1046	F
Benzyl benzoate	1738	F
α-BHC	1690**	E
β-BHC	1710**	E
δ-BHC	1755**	E
Biphenyl	1389	I
4,4-Bipyridyl dihydrate	1507	I
Bisoflex 1001***	MP	P
1-Bromodecane	1326	I
Bromonaphthalene	1434	I
Bulan***	2310**	E
2-Butoxyethyl oleate	MP	P
Butylated hydroxyanisole	1462	A
Butylated hydroxytoluene	1490	A
Butylbenzyl phthalate	2327	P
Butylbenzyl sebacate	1585	P
	2130	
	2520	
Butyl epoxystearate	MP	P
Butylisodecyl phthalate	1950	P
Butyl oleate	MP	P
Butyl-PBD	3342	S
Butylsextyl phthalate	1940	P
	2235	
Butyl stearate	2157	P
	2362	
Cadaverine	1035	D
Camphor	1137	P
Captan	2000**	E

TABLE 2 *(continued)*

Compound	*Retention index*	*Classification*
Carbazole	1784	I
Carbophenothion	2255**	E
Castor oil	MP	P
CDEC	1685**	E
Chlorobenside	2040	E
Chlordecone	2240**	E
o-Chlorobenzylidenemalonitrile	1516	E
5*α*-Cholestane	2852	I
Cholesterol	3008	D
Choline	NO	D
Citral	1272	F
Citroflex A4***	2224	P
Cresylphenyl phosphate	MP	P
Cyclododecanone	1524	E
Cyclohexylisooctyl phthalate	2446 2532	P
Cyclohexyltridecyl phthalate	2518	P
2,4-D butyl ester	1840**	E
2,4-D isobutyl ester	1805**	E
2,4-D isopropyl ester	1700**	E
2,4-D methyl ester	1605**	E
DCPA	1960**	E
DDA methyl ester	2085**	E
o,p'-DDE	2070**	E
p,p'-DDE	2130**	E
o,p'-DDT	2220**	E
p,p'-DDT	2290*	E
Demeton-S-methyl	1628	E
Dialkyl adipate 79***	MP	P
Diallyl phthalate	1698	P
Dialphanol phthalate	MP	P
Dialphanol sebacate	MP	P
Diamyl phthalate	2140	P
Diazinon	1760*	E
Dibenzyl phthalate	2690	P
Dibenzyl sebacate	2135	P
m-Dibromobenzene	1197	I
o-Dibromobenzene	1221	I
p-Dibromobenzene	1193	I
p-Dibutoxyethoxyethyl adipate	1285	P
Di-(butoxyethyl) phthalate	2850	P
Di-(butoxyethyl) sebacate	2700	P
Dibutyl adipate	1660	P
Dibutyl maleate	1505	P
Di-*n*-butyl phthalate	1924	P
Dibutyl sebacate	2137	P
Di-*n*-butyl terephthalate	2066	P
Dibutyltin dilaurate	NO	P
Dichlone	1760**	E
p-Dichlorobenzene	1000	E
Dicyclohexyl adipate	2282	P
Dicyclohexyl oxalate	1880	P

(Continued on p. 190)

TABLE 2 *(continued)*

Compound	*Retention index*	*Classification*
Dicyclohexyl phthalate	2461	P
Dieldrin	2100**	E
Di(ethoxyethyl) adipate	1880	P
Di(ethoxyethyl) phthalate	2135	P
Di(ethoxyethyl) sebacate	2270	P
Diethyl adipate	1349	P
Di(2-ethylhexyl) adipate	2381	P
Di(2-ethylhexyl) isophthalate	2730	P
Di(2-ethylhexyl) phthalate	2507	P
Di(2-ethylhexyl) sebacate	2792	P
Diethyl maleate	1081	P
Diethyl phthalate	1568	P
Diethyl sebacate	1746	P
N,N-Diethyl-N,N-toluamide	1571	E
Diheptyl phthalate	2500	P
9,10-Dihydroanthracene	1662	I
Diisobutyl adipate	1660	P
Diisobutyl phthalate	1863	P
Diisobutyl terephthalate	1972	P
Diisodecyl adipate	2745	P
Diisodecyl phthalate	2511	P
Diisoheptyl phthalate	MP	P
Diisooctyl adipate	MP	P
Diisooctyl epoxystearate	MP	P
Diisooctyl maleate	MP	P
Diisooctyl phthalate	MP	P
Diisooctyl sebacate	MP	P
Dimethoate	1720	E
Dimethoxyethyl phthalate	1980	P
Dimethyl adipate	1223	P
p-Dimethylaminobenzaldehyde	1528	I
Dimethyl isophthalate	1488	P
α,α-Dimethyl-β-methylsuccinamide	1195	I
Dimethyl phthalate	1434	P
Dimethyl POPOP	3618	S
2,4-Dimethyl quinoline	1446	I
2,7-Dimethyl quinoline	1425	I
Dimethyl sebacate	1645	P
Dimethyl terephthalate	1475	P
Dinonyl adipate	2484	P
Dinonyl phthalate	2649	P
Dioctyl adipate	2383	P
Dioctyl phthalate	2519	P
Dioctyl sebacate	2782	P
Di(*n*-decyl) adipate	2905	P
Diphenyl adipate	2397	P
Diphenyl mercury	1873	P
Diphenyl phthalate	2550	P
Dipropyl adipate	1545	P
Dipropyl phthalate	1743	P
Disextyl maleate	2116	P
Ditridecyl phthalate	NO	P
Dyrene	2010**	E

TABLE 2 *(continued)*

Compound	*Retention index*	*Classification*
Endosulfan I	2085**	E
Endosulfan II	2175**	E
Endrin	2165	E
Ethanolamine	780	D
Ethion	2220**	E
Ethylamine	NO	D
Ethylan	2175	E
Ethylbenzoate	1227	F
Ethyl oleate	2175	P
5-Ethyl-5-*p*-tolyl barbituric acid	2085	I
Eugenol	1368	F
Fluorene	1580	I
Fluorenone	1705	I
Folpet	2015**	E
Glyceryl dibenzoate	2442	P
Glycerol	NO	P
Harman	2000	D
Heptachlor	1890**	E
Heptachlor epoxide	2015**	E
Hexaphenyl benzene	NO	S
Howflex GBP***	1947	P
1-Hydroxychlordene	1955**	E
1-Hydroxyharman	1920	D
	2015	
	2290	
Imidazole	1095	D
Indene	1062	I
Indole	1276	D
Isatin	1712	I
Isobutylcyclohexyl phthalate	1868	P
	2159§	
	2453	
3-Isobutyl-1-methyl xanthine	2150	I
Isobutylsextyl phthalate	MP	P
Isoheptylcyclohexyl phthalate	MP	P
Isooctyl epoxystearate	MP	P
Isooctylisodecyl phthalate	MP	P
Lankroflex 79LP***	MP	P
Lankroflex 79LTM***	MP	P
Limonene	1053	F
Lindane	1757*	E
Linevol 79 phthalate***	MP	P
Linevol 911 phthalate***	MP	P
Linseed oil	MP	P
Liquid paraffin	MP	P
Malathion	1900*	E
2-Mercaptobenzoxazole	NO	P
2-Mercaptobenzimidazole	NO	P
2-Mercaptobenzothiazole	1936	P
Methoxychlor	2410*	E

(Continued on p. 192)

TABLE 2 *(continued)*

Compound	*Retention index*	*Classification*
Methyl anthranilate	1343	I
Methyl caprylate	1130	I
Methyl decanoate	1305	D
Methyldiphenyl phosphate	MP	P
Methyl *p*-hydroxybenzoate	1419	A
Methyl linoleate	2100	D
2-Methylnaphthalene	1313	I
Methyl nonanoate	1215	D
Methyl oleate	2086	P
Methyl palmitate	1867	D
5-Methyl-5-phenylhydantoin	1866	I
5(*p*-Methylphenyl)-5-phenylhydantoin	2457	I
Methyl stearate	2116	P
Mevinphos	1450**	E
Mirex	2470**	E
Monocresyldiphenyl phosphate	MP	P
Morpholine	810	D
Naphthalene	1186	S
1-Naphthonitrile	1489	I
2-Naphthyl acetate	1585	I
Nicotinamide	1475	D
Norharman	2005	D
Octyldecyl adipate	2540 2745 2940	P
4-*tert*.-Octyl-2-methyl-cyclohexyl acetate	1611	P
Oleamide	MP	P
Parathion	1935**	E
Parathion methyl	1845	E
Perthane	2175*	E
Phenazine	1703	I
1-Phenylethylamine	1050	D
2-Phenylethylamine	1125	D
Phorate	1675**	E
Piperidine	790	D
Pliabrac A***	MP	P
Pliabrac 519***	MP	P
Pliabrac 521***	MP	P
Pliabrac 524***	MP	P
Pliabrac 985***	MP	P
Pliabrac 987***	MP	P
Pliabrac 989***	MP	P
Pliabrac 990***	MP	P
POPOP	3525	S
PPO	2050	S
Prazepam	2610	I
Prolan	2250**	E
Propylene glycol adipate	MP	P
Propyl-*p*-hydroxybenzoate	1567	A
Putrescine	930	D
Pyrene	1983	I
Pyribenzamine	1980	I
Pyrrolidine	695	D
Quinoline	1247	I
Ronnel	1880**	E

TABLE 2 *(continued)*

Compound	*Retention index*	*Classification*
Sancticiser 141***	2410[§]	P
Sancticiser 148***	MP	P
SKF 525A***	2326	I
Simazine	1690**	E
Stigmasterol	3234	I
trans-Stilbene	1755	I
2,4,5-T isopropyl ester	1825**	E
2,4,5-T methyl ester	1740**	E
TDE *o,p'* isomer	2130	E
TDE *p,p'* isomer	2200	E
p-Terphenyl	2208	I
Terpineol (mixed isomers)	1127	F
	1170	
	1183	
Tetrachlorvinphos *Z* isomer	2084	E
Tetradifon	2430**	E
Tetrahydrofurfuryl oleate	2290	P
	2480	
	2660	
Tetraphenylethylene	2478	I
Thianaphthene	1200	I
Thianthrene	1901	P
Triacetin	1282	P
Tributyrin	1552	P
Tri(2-chloroethyl) phosphate	1740	P
Tribenzylamine	2271	I
Tributyl citrate	2150	P
Tributyl phosphate	1690	P
Tricresyl phosphate	2695	P
Triethyl citrate	1655	P
Tri(2-ethylhexyl) phosphate	2463	P
Triethyl phosphate	1109	P
Triflubazam	2244	I
Triisobutyl phosphate	1483	P
Triisopropyl phosphate	1182	P
Trimethyl citrate	1442	P
Trimethyl phosphate	995	P
Trioctyl phosphate	2445	P
Triolein	NO	P
Triphenylamine	2055	I
Triphenyl phosphate	2363	P
Tripropyl phosphate	1372	P
Triptylene	2224	I
Tris(butoxyethyl) phosphate	2363	P
Tris(2,3,-dibromo-propyl) phosphate	NO	P
Tris(2,3,-dichloropropyl) phosphate	2307	P
Tris(isopropylphenyl) phosphate	MP	P
Tritolyl phosphate	MP	P
Trixylenyl phosphate	MP	P
Tryptamine	1750	D
Tyramine	1405	D
Uracil	NO	D
Xanthine	NO	I

* From ref. 16.
** From ref. 18.
*** Trade name.
§ Major peak.

TABLE 3
RETENTION INDICES OF 253 COMPOUNDS, USING SE-30 OR OV-1 AS THE STATIONARY PHASE, ARRANGED IN ASCENDING ORDER OF RETENTION INDEX
NO = No peak observed; MP = multiple peak, *i.e.* more than three.

Compound	*Retention index*	*Classification*
Acetylcholine	NO	D
Adenine	NO	D
Choline	NO	D
Dibutyltin dilaurate	NO	P
Ditridecyl phthalate	NO	P
Ethylamine	NO	D
Glycerol	NO	P
Hexaphenyl benzene	NO	S
2-Mercaptobenzimidazole	NO	P
2-Mercaptobenzoxazole	NO	P
Triolein	NO	P
Tris(2,3-dibromopropyl) phosphate	NO	P
Uracil	NO	D
Xanthine	NO	I
Pyrrolidine	695	D
Ethanolamine	780	D
Piperidine	790	D
Morpholine	810	D
Putrescine	930	D
Trimethyl phosphate	995	P
p-Dichlorobenzene	1000	E
Cadaverine	1035	D
Benzyl alcohol	1046	F
1-Phenylethylamine	1050	D
Limonene	1053	F
Indene	1062	I
Diethyl maleate	1081	P
Imidazole	1095	D
Triethyl phosphate	1109	P
2-Phenylethylamine	1125	D
Terpineol (mixed isomers)	1127	F
	1170	
	1183	
Methyl caprylate	1130	I
Camphor	1137	P
3-Amino-2-dimethylbenzene	1178	I
Triisopropyl phosphate	1182	P
Naphthalene	1186	S
p-Dibromobenzene	1193	I
α,α-Dimethyl-β-methyl succinamide	1195	I
m-Dibromobenzene	1197	I
Thianaphthene	1200	I
Methyl nonanoate	1215	D
o-Dibromobenzene	1221	I
Dimethyl adipate	1223	P
Ethylbenzoate	1227	F
Quinoline	1247	I
Citral	1272	F
Indole	1276	D
Triacetin	1282	P

TABLE 3 *(continued)*

Compound	*Retention index*	*Classification*
Anethole	1284	F
p-Dibutoxyethoxyethyl adipate	1285	P
Methyl decanoate	1305	D
2-Methylnaphthalene	1313	I
1-Bromodecane	1326	I
Methyl anthranilate	1343	I
Diethyl adipate	1349	P
Eugenol	1368	F
Tripropyl phosphate	1372	P
Biphenyl	1389	I
Tyramine	1405	D
Methyl *p*-hydroxybenzoate	1419	A
2,7-Dimethylquinoline	1425	I
Bromonaphthalene	1434	I
Dimethyl phthalate	1434	P
Trimethyl citrate	1442	P
2,4-Dimethylquinoline	1446	I
Mevinphos	1450**	E
Butylated hydroxyanisole	1462	A
Dimethyl terephthalate	1475	P
Nicotinamide	1475	D
Triisobutyl phosphate	1483	P
Dimethyl isophthalate	1488	P
1-Naphthonitrile	1489	I
Butylated hydroxytoluene	1490	A
Dibutyl maleate	1505	P
4,4-Bipyridyl dihydrate	1507	I
o-Chlorobenzylidenemalonitrile	1516	E
Cyclododecanone	1524	E
p-Dimethylaminobenzaldehyde	1528	I
Dipropyl adipate	1545	P
Tributyrin	1552	P
Azobenzene	1556	I
Propyl-*p*-hydroxybenzoate	1567	A
Diethyl phthalate	1568	P
N,N-Diethyl-N,N-toluamide	1571	E
Fluorene	1580	I
2-Naphthyl acetate	1585	P
Butylbenzyl sebacate	1585	P
	2130	
	2520	
5-Aminoquinoline	1598	I
2,4-D methyl ester	1605**	E
Benzophenone	1611	F
4-*tert.*-Octyl-2-methyl-cyclohexyl acetate	1611	P
Demeton-S-methyl	1628	E
Dimethyl sebacate	1645	P
Triethyl citrate	1655	P
Dibutyl adipate	1660	P
Diisobutyl adipate	1660	P
9,10-Dihydroanthracene	1662	I
Phorate	1675**	E

(Continued on p. 196)

TABLE 3 *(continued)*

Compound	*Retention index*	*Classification*
CDEC	1685**	E
α-BHC	1690**	E
Tributyl phosphate	1690	P
Simazine	1690**	E
Diallyl phthalate	1698	P
2,4-D isopropyl ester	1700**	E
Phenazine	1703	I
Atrazine	1705**	E
Fluorenone	1705	I
β-BHC	1710**	E
Anthracene	1711	I
Isatin	1712	I
Dimethoate	1720**	E
Acetyltriethyl citrate	1730	P
Benzyl benzoate	1738	F
2,4,5-T methyl ester	1740**	E
Tri(2-chloroethyl) phosphate	1740	P
Dipropyl phthalate	1743	P
Diethyl sebacate	1746	P
Tryptamine	1750	D
δ-BHC	1755**	E
trans-Stilbene	1755	I
Lindane	1757*	E
Diazinon	1760*	E
Dichlone	1760	E
Carbazole	1784	I
2,4-D isobutyl ester	1805**	I
2,4,5-T isopropyl ester	1825**	E
2,4-D butyl ester	1840**	E
Parathion methyl	1845	E
Diisobutyl phthalate	1863	P
5-Methyl-5-phenylhydantoin	1866	I
Methyl palmitate	1867	D
Isobutylcyclohexyl phthalate	1868	P
	2159§	
	2453	
Diphenyl mercury	1873	P
Dicyclohexyl oxalate	1880	P
Di(ethoxyethyl) adipate	1880	P
Ronnel	1880**	E
Heptachlor	1890**	E
Malathion	1900*	E
Thianthrene	1901	I
1-Hydroxyharman	1920	D
	2015	
	2290	
Di-*n*-butyl phthalate	1924	P
Parathion	1935**	E
2-Mercaptobenzothiazole	1936	P
Butylsextyl phthalate	1940	P
	2235	
Howflex GBP***	1947	P
Aldrin	1950*	E
Butylisodecyl phthalate	1950	P
1-Hydroxychlordene	1955**	E
DCPA	1960**	E
Diisobutyl terephthalate	1972	P
Dimethoxyethyl phthalate	1980	P

TABLE 3 *(continued)*

Compound	*Retention index*	*Classification*
Pyribenzamine	1980	I
Pyrene	1983	I
Captan	2000**	E
Harman	2000	D
Norharman	2005	D
Anilazine	2010	E
Dyrene	2010**	E
Folpet	2015**	E
Heptachlor epoxide	2015**	E
Chlorobenside	2040**	E
PPO	2050	S
Triphenylamine	2055	I
Di-*n*-butyl terephthalate	2066	P
o,p-DDE	2070**	E
Tetrachlorvinphos *Z* isomer	2084	E
DDA methyl ester	2085**	E
Endosulfan I	2085**	E
5-Ethyl-5-*p*-tolyl barbituric acid	2085	I
Methyl oleate	2086	P
Dieldrin	2100*	E
Methyl linoleate	2100	D
Disextyl maleate	2116	P
Methyl stearate	2116	P
p,p'-DDE	2130**	E
TDE *o,p'* isomer	2130	E
Di(ethoxyethyl) phthalate	2135	P
Dibenzyl sebacate	2135	P
Dibutyl sebacate	2137	P
Diamyl phthalate	2140	P
3-Isobutyl-1-methyl xanthine	2150	I
Tributyl citrate	2150	P
Butyl stearate	2157	P
	2362	
Endrin	2165**	E
Endosulfan II	2175**	E
Ethylan	2175	E
Ethyl oleate	2175	P
Perthane	2175*	E
TDE *p,p'* isomer	2200	E
p-Terphenyl	2208	I
o,p'-DDT	2220**	E
Ethion	2220**	E
Citroflex A4***	2224	P
Triptylene	2224	I
Chlordecone	2240**	E
Triflubazam	2244	I
Prolan	2250**	E
Acetyltributyl citrate	2253	P
Carbophenothion	2255**	E
Di(ethoxyethyl) sebacate	2270	P
Tribenzylamine	2271	I
Dicyclohexyl adipate	2282	P
9,10-Anthracenedicarbonitrile	2288	I
p,p'-DDT	2290*	E
Tetrahydrofurfuryl oleate	2290	P
	2480	
	2660	

(Continued on p. 198)

TABLE 3 *(continued)*

Compound	*Retention index*	*Classification*
Tris(2,3-dichloropropyl)phosphate	2307	P
Bulan***	2310**	E
SKF 525A***	2326	I
Butylbenzyl phthalate	2327	P
Triphenyl phosphate	2363	P
Tris(butoxyethyl) phosphate	2363	P
Di(2-ethylhexyl) adipate	2381	P
Dioctyl adipate	2383	P
Diphenyl adipate	2397	P
Sancticiser 141***	2410§	P
Methoxychlor	2410*	E
Azinphosmethyl	2430**	E
Tetradifon	2430**	E
Glyceryl dibenzoate	2442	P
Trioctyl phosphate	2445	P
Cyclohexylisooctyl phthalate	2446	P
	2532	
5(*p*-Methylphenyl)-5-phenylhydantoin	2457	I
Dicyclohexyl phthalate	2461	P
Tri(2-ethylhexyl) phosphate	2463	P
Mirex	2470**	E
Tetraphenylethylene	2478	I
Dinonyl adipate	2484	P
Diheptyl phthalate	2500	P
Di(2-ethylhexyl) phthalate	2507	P
Diisodecyl phthalate	2511	P
Cyclohexyltridecyl phthalate	2518	P
Dioctyl phthalate	2519	P
Octyldecyl adipate	2540	P
	2745	
	2940	
Diphenyl phthalate	2550	P
Prazepam	2610	I
Dinonyl phthalate	2649	P
Dibenzyl phthalate	2690	P
Tricresyl phosphate	2695	P
Di(butoxyethyl)sebacate	2700	P
Di(2-ethylhexyl) isophthalate	2730	P
Di(isodecyl) adipate	2745	P
BBOT	2745	S
	3750§	
Dioctyl sebacate	2782	P
Di(2-ethylhexyl) sebacate	2792	P
Di(butoxyethyl) phthalate	2850	P
5α-Cholestane	2852	I
Di(*n*-decyl) adipate	2905	P
Cholesterol	3008	D
Stigmasterol	3234	I
Butyl PBD	3342	S
POPOP	3525	S
Dimethyl POPOP	3618	S
BBO	3710	S

* From ref. 16.
** From ref. 18.
*** Trade name.
§ Major peak.

phase DC-200 expressed as retention ratios relative to aldrin. Seven data points from this collection were plotted as log retention ratios against known retention indices[16]. The regression obtained was:

$$\text{Retention index on SE-30} = 706\ (\text{log retention ratio}) + 1955$$

with a correlation coefficient of 0.9993. The rest of the data were then transformed into retention indices on SE-30 using the equation above and included in Tables 2 and 3. The transformation of retention times, retention ratios and retention indices using the same, or closely related, stationary phases is therefore an easy way to compile standardised data without the need to rechromatograph every substance.

The use of retention indices for standardisation in GLC has enabled compilations of data to be made for use in toxicological analyses[13,14,16,21]. For these collections to be of the greatest use, it is imperative that the factors affecting the reproducibility of retention indices are recognised and precautions taken to minimise the errors of measurement. The choice of support is an important feature[22] and in general it should be inactivated as thoroughly as possible. The effects of the residual active sites may be reduced by using a high-polarity phase or by using a sufficiently high loading of stationary phase[22,23]. High-polarity phases unfortunately give poorer reproducibility than low-polarity phases[9] so that a good compromise is between 2 and 5% of a low-polarity phase. The temperature is another very important feature and it has been shown by several authors that the retention index is dependent on the temperature of the column[21,24,25] so that the measurement of very short or very long retention times should be avoided. Thus, to obtain reproducible results the conditions used for chromatography should not be too far removed from those used by the workers who compiled the data. The sample size is important[26] since longer retention times will be observed for polar materials as the quantity chromatographed is decreased.

In order to compensate for different conditions in different laboratories (*e.g.* support, different phase loadings, temperature, etc.) Moffat[16] suggested that an error factor of $\pm$ 50 retention index units would be an acceptable limit of reproducibility for measurements based on a standard deviation of 18 retention index units. To measure the reproducibility of results between our two laboratories, 17 compounds were randomly selected from the various chemical classes of substances in this collection and retention indices of these compounds were measured independently in each laboratory. Despite the fact that a 4 m $\times$ 3 mm I.D. column of 3% (w/w) OV-1 on Chromosorb W HP was used in one laboratory and a 1.5 m $\times$ 4 mm I.D. column of 3% (w/w) SE-30 on Chromosorb G HP was used in the second laboratory, the mean interlaboratory difference between indices for the 17 compounds was $\pm$ 13 retention index units, with only one outside the $\pm$ 50 limit (difference of 55 units). This supports the claims that the use of retention index measurements provides a good basis for peak identification procedures.

For a more rigorous identification of an ester, it is possible to use the method suggested by Krishen[1]. This involves the hydrolysis of the ester, and subsequent chromatography of the alcohol and the acid after it has been methylated.

A total of 14 compounds did not elute under the conditions used in this work. However, the majority of compounds eluted as single peaks, although a few excep-

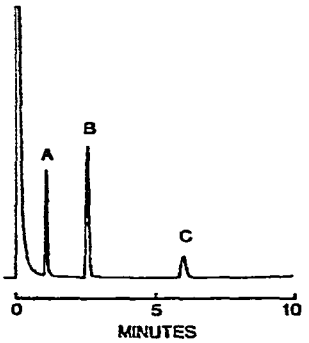

Fig. 1. Chromatogram of a commercial sample of isobutylcyclohexyl phthalate using FID. A, diisobutyl phthalate; B, isobutylcyclohexyl phthalate; C, dicyclohexyl phthalate.

tions were noted. The retention indices of the 8 compounds giving up to three significant peaks have been incorporated into Tables 2 and 3. In most cases, where more than one major peak was observed, the identity of the extra peaks reflected the mode of synthesis of the supposedly primary compound. For example, three peaks were observed in the chromatogram of isobutylcyclohexyl phthalate (Fig. 1) which were identified by mass spectrometry as diisobutyl phthalate, isobutylcyclohexyl phthalate and dicyclohexyl phthalate. Other examples are given in Table 4. Some compounds (a total of 44) revealed even more complex chromatograms. In cases where more than three significant peaks were observed, the compound was listed in Table 2 as having multiple peaks and omitted from Table 3. A list of compounds chromatographing as multiple peaks is presented in Table 5 and an example is shown in Fig. 2. The complex nature of some of these substances may be indicated by a "noisy baseline" rather than by distinct peaks.

TABLE 4

IDENTIFICATION OF SOME COMPOUNDS EXHIBITING MULTIPLE PEAKS BY GAS–LIQUID CHROMATOGRAPHY

Compound	*Peak identification (by mass spectrometry)*
Octyldecyl adipate	Octyl adipate
	Dioctyl adipate
	Octyldecyl adipate
Cresylphenyl phosphate	Diphenylcresyl phosphate
	Dicresylphenyl phosphate
	Tricresyl phosphate
Isobutylcyclohexyl phthalate	Diisobutyl phthalate
	Dicyclohexyl phthalate
	Isobutylcyclohexyl phthalate
Butylbenzyl sebacate	Dibutyl sebacate
	Butylbenzyl sebacate
	Dibenzyl sebacate
Isooctylisodecyl phthalate	Diisooctyl phthalate
	Isooctylisodecyl phthalate
	Diisodecyl phthalate

TABLE 5

ALPHABETICAL LIST OF COMPOUNDS ELUTING AS MULTIPLE PEAKS (*i.e.* MORE THAN THREE)

Compound	*Classification*
Alphanol 610*	P
Barkite B	P
Bisoflex 1001*	P
2-Butoxyethyl oleate	P
Butyl epoxystearate	P
Butyl oleate	P
Castor oil	P
Cresylphenyl phosphate	P
Dialkyl adipate 79*	P
Dialphanol phthalate	P
Dialphanol sebacate	P
Diisoheptyl phthalate	P
Diisooctyl phthalate	P
Diisooctyl adipate	P
Diisooctyl epoxystearate	P
Diisooctyl maleate	P
Diisooctyl phthalate	P
Diisooctyl sebacate	P
Isobutylsextyl phthalate	P
Isoheptylcyclohexyl phthalate	P
Isooctyl epoxystearate	P
Isooctylisodecyl phthalate	P
Lankroflex 79LP*	P
Lankroflex 79LTM*	P
Linevol 79 phthalate*	P
Linevol 911 phthalate*	P
Linseed oil	P
Liquid paraffin	P
Methyldiphenyl phosphate	P
Monocresyldiphenyl phosphate	P
Oleamide	P
Pliabrac A*	P
Pliabrac 519*	P
Pliabrac 521*	P
Pliabrac 524*	P
Pliabrac 985*	P
Pliabrac 987*	P
Pliabrac 989*	P
Pliabrac 990*	P
Propylene glycol adipate	P
Sancticiser 148*	P
Tris(isopropylphenyl) phosphate	P
Tritolyl phosphate	P
Trixylenyl phosphate	P

* Trade name.

3.2. Examples of interferences by plasticizers

Plasticizers and other non-drug substances may enter biological samples from a variety of sources or may even be added inadvertently during analysis. For example, in a vertical injection system particles of a septum may be dislodged on to the top

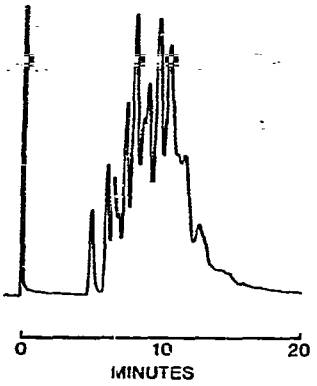

Fig. 2. Chromatogram of a commercial sample of tritolyl phosphate using PND in phosphorus mode.

of the column and if the septum contains a plasticizer, peaks for this compound may be observed. An illustration of the presence of a plasticizer in a GLC septum with similar chromatographic properties to tris(isopropylphenyl) phosphate is shown in Fig. 3.

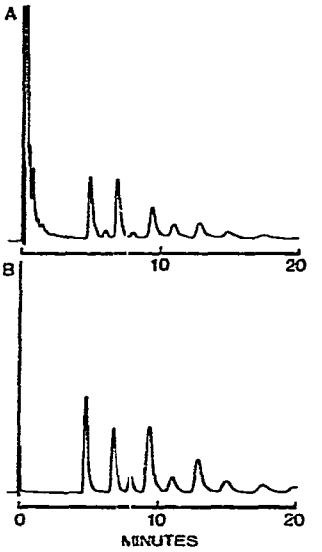

Fig. 3. Chromatograms obtained using PND in phosphorus mode of A, an acetone extract of a GLC septum; B, tris(isopropylphenyl) phosphate.

In addition to the retention index data, the properties of substituents in the molecules may be of aid in the identification of unknown peaks since in many instances the response ratio of compounds between different selective detectors can be characteristic[27]. The FID response is dependent on the number of carbon atoms in a molecule and is quite predictable. However, the ECD response for different compounds varies widely and is dependent on the electron deficient part of the compound, and is quite difficult to predict. The PND response for a compound depends to some extent on

the number of nitrogen or phosphorus atoms in a molecule but also depends on their environment. It follows that by using the FID as a reference we can measure the ECD or PND response relative to it, thus adding another variable to measure in addition to the retention index.

The ECD is very sensitive to phthalate esters. For example, Fig. 4 illustrates the presence of di(2-ethylhexyl) phthalate (DEHP) as an impurity in a blood extract from a patient who had received a transfusion of blood which had been stored in a plastic bag. The ECD/FID response ratio was of valuable assistance in the differentiation between plasticizer peaks and the compounds of interest (benzodiazepines) since the ECD/FID ratio for DEHP differs considerably from that of benzodiazepines.

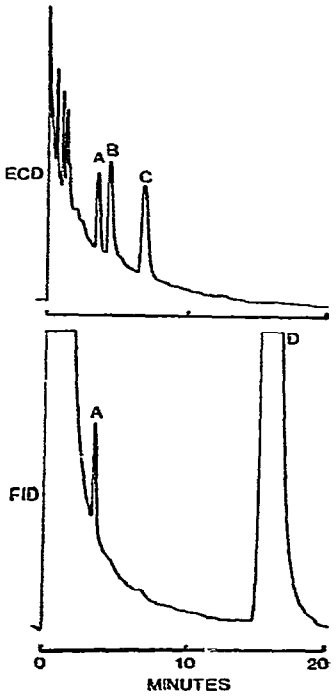

Fig. 4. Chromatograms, using both FID and ECD, of an extract of a blood sample from a patient taking flurazepam and who had had a blood transfusion. A, di(2-ethylhexyl) phthalate from the poly-(vinylchloride) transfusion bag; B, desalkylflurazepam (a metabolite of flurazepam); C, prazepam (internal standard); D, cholesterol.

The heated bead PND may be made very highly selective for phosphorus containing compounds, but will retain its sensitivity to phosphorus even when optimised for its nitrogen response. Hence, by running a chromatogram with the PND optimised for phosphorus and then running the chromatogram with the detector optimised for nitrogen it may be deduced if a compound contains nitrogen or phosphorus. In addition, if a dual-detector system (PND/FID) is used, the greater sensitivity of the PND to phosphorus containing compounds compared with nitrogen

containing compounds can be recognised using the FID response as a reference. Fig. 5 illustrates the use of the PND/FID response ratios to reveal the phosphate impurities originating from filter paper present in a blood extract containing barbiturates (which contain nitrogen).

Tables 2 and 3 contain retention data for compounds that have been used as internal standards for the quantitative analyses of drugs. Because of the insidious nature of plasticizers, as shown by the above examples, it is obvious that their use as internal standards may lead to errors.

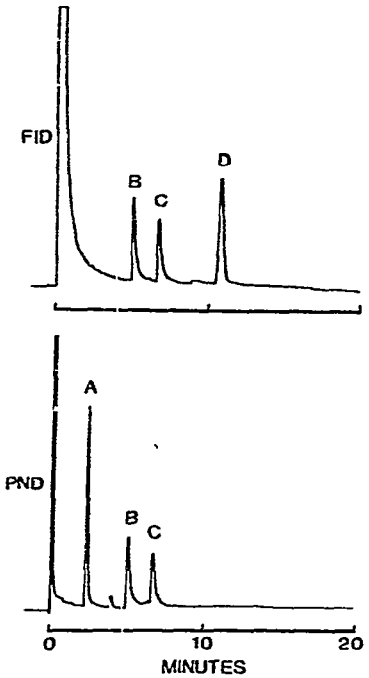

Fig. 5. Chromatograms using FID and PND in nitrogen mode, with attenuations adjusted to give roughly equal sized peaks for the barbiturates on both detectors, of an extract of a blood sample containing barbiturates. The contamination from triisobutyl phosphate introduced from filter paper is apparent. A, triisobutyl phosphate; B, amylobarbitone; C, quinalbarbitone; D, C_{19} H_{40} (retention index marker).

4. CONCLUSIONS

Plasticizers and other non-drug substances may find their way into analytical extracts via a number of different routes. Hence the presence of these impurities may provide analytical complications to the unsuspecting analyst. The retention indices of 296 non-drug substances have been measured on SE-30 or OV-1 to supplement collections of previously published data on these non-polar stationary phases. The use of selective GLC detector response ratios may, in many instances, be of value in differentiating between drug and non-drug substances in biological extracts. The use of plasticizers as internal standards for quantitative analyses is to be discouraged.

5. ACKNOWLEDGEMENTS

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6. SUMMARY

The advent of the widespread use of selective detectors (electron capture detector, phosphorus/nitrogen detector) for gas–liquid chromatography used in toxicological analyses has revealed the presence of hitherto unseen interfering materials. These substances may be conveniently grouped into (1), anti-oxidants; (2), putrefactive and endogenous compounds; (3), pesticides; (4), food additives, flavours and fragrances; (5) plasticisers, plastic additives and vulcanising agents and (6), scintillation reagents. To facilitate the identification of these materials, retention indices on the dimethyl silicone phases SE-30 or OV-1 have been compiled by the two laboratories to include 296 such compounds. Most gave single peaks, but some gave complex patterns indicating that they were mixtures of compounds. Of the 296 compounds, 14 did not give observable peaks, 8 gave 2 or 3 peaks and 44 gave more than 3 peaks. To determine the interlaboratory difference between retention index measurements, 17 compounds were chromatographed by both laboratories: the mean difference was $\pm$ 13 retention index units with only one greater than $\pm$ 50 retention index units. Examples of how these materials may be encountered during toxicological analyses are given. Data are also presented on compounds which have been used as internal standards.

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