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Note

Gas–liquid chromatographic retention of phthalates and their transesterified products in terms of equivalent chain length values of fatty acid methyl esters

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Phthalates are used extensively as plasticizers in the formulation of polymers[1,2]. Because plasticizers are not chemically bounded to the polymer, they can migrate from the plastic into the environment under certain conditions[3]. Phthalates have been found as contaminants in many samples of biological origin and foods such as human blood[4], egg[5], milk[6], fish[7] and water[2,3]. It has been also found that phthalates migrate from plastics into chemicals, solvents and laboratory equipment[8,9]. Because of the apparent facility with which these esters are leached from a variety of plastic materials and containers, stringent precautions are required in analyses of fatty acids of lipid samples that closely match the gas chromatographic behaviour of phthalates. On the other hand, during the transesterification of O-acyl lipids for the preparation of fatty acid methyl esters, phthalate contaminants were also transesterified to form various methyl ester derivatives of orthophthalic acid and alcohols[10]. The possibility that phthalates and their transesterified products are present among fatty acid methyl ester preparations from O-acyl lipids creates obvious problems in the identification and determination of fatty acids by gas–liquid chromatography (GLC).

In this paper, we report the retention indices of phthalates and their transesterified products, in terms of equivalent chain length (ECL) values of fatty acid methyl esters, obtained by GLC on seven stationary phases that are commonly used in the analysis of fatty acid methyl esters.

EXPERIMENTAL

Materials

Fourteen phthalates (see Table II) were purchased from Tokyo Kasei (Tokyo, Japan). All solvents (analytical-reagent grade) were purchased from Wako (Tokyo, Japan) and were distilled before use.

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Gas–Liquid chromatographic conditions

The experiments were carried out on a Hitachi Model 163 gas chromatograph equipped with a flame-ionization detector. Stainless-steel columns (2 m × 3 mm I.D.) were used throughout. The samples were analysed on the following seven columns: (1) 8% diethylene glycol succinate polyester (DEGS) operated at 180°C; (2) 3% SE-30 at 230°C; (3) 2% OV-17 230°C; (4) 1.5% SE-52 at 180°C; (5) 3% QF-1 at 230°C; (6) 10% PEG-20M at 230°C; and (7) 10% Apiezon M at 250°C. The injector and detector temperatures were 250 and 280°C, respectively, and the carrier gas (nitrogen) flow-rate was 30 ml/min.

Transesterification of phthalates

O-Acyl lipids were transesterified by heating them with a large excess of anhydrous methanol in the presence of an acidic catalyst. The commonest and mildest reagent used for this purpose was 3–5% anhydrous hydrogen chloride in methanol. The same method was used to prepare transesterification products of phthalates; the phthalate sample (about 5 mg) was dissolved in 3% HCl–methanol (2 ml) in a vial with a PTFE screw-cap, heated at 90°C for 2 h, then water (2 ml) containing sodium chloride (15%) was added and the products were extracted with diethyl ether. After the diethyl ether layer had been dried over anhydrous sodium sulphate, an aliquot was injected into the GLC system.

Equivalent chain length (ECL) values

GLC retention indices of phthalates and their transesterification products in terms of the ECL of fatty acid methyl esters were found by reference to the straight line obtained by plotting the logarithms of the retention times of a homologous series of straight-chain saturated fatty acid methyl esters against the number of carbon atoms in the aliphatic chain of each acid. The retention times of phthalates and their transesterification products were measured under identical operating conditions and the ECL values were read directly from the graph.

RESULTS AND DISCUSSION

Gas chromatogram of transesterification products of phthalate

Fig. 1 shows a typical gas chromatogram of the transesterification products of di-2-ethylhexyl phthalate (DEHP) on an OV-17 column. Four peaks for 2-ethylhexanol, dimethyl phthalate (DMP), methyl-2-ethylhexyl phthalate and untransesterified DEHP were clearly resolved. It is concluded that dialkyl phthalates are transesterified by the acid catalyst very slowly to produce methyl alkyl and dimethyl phthalate, as shown in Scheme I.

Typical relationships between the reaction time and the relative concentration at 90°C of the products from di-*n*-butyl phthalate (DBP) are shown in Fig. 2. The effect of different catalysts on the transesterification of DBP and DEHP are given in Table I. The rate of transesterification of DEHP was lower than that of DBP under the same conditions.

ECL values of phthalates and their methyl esters

The ECL values of commercial phthalates and their methyl esters on seven

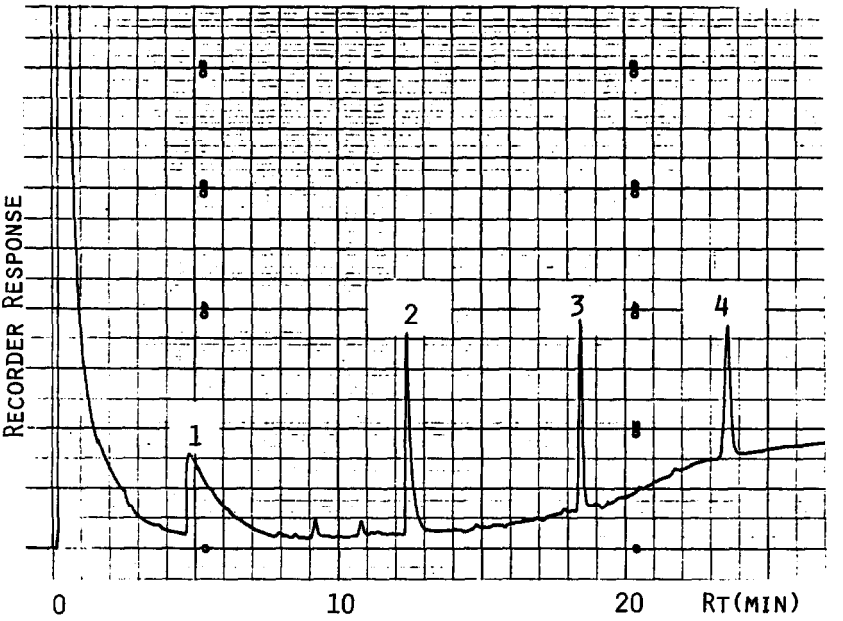
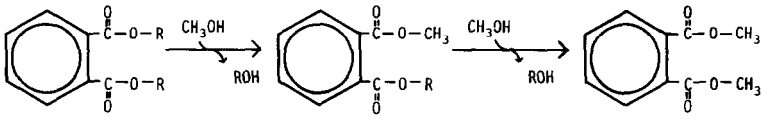


Fig. 1. Typical gas chromatogram of transesterification products of DEHP. GLC conditions: column, 2.0% OV-17; column temperature, programmed from 50 to 210°C at 5°C/min. Peaks: 1 = 2-ethylhexanol; 2 = dimethyl phthalate (DMP); 3 = methyl 2-ethylhexyl phthalate; 4 = di-2-ethylhexyl phthalate (DEHP).

columns are given in Table II. The ECL values were in the range 16.26–22.27 for DBP, 13.67–20.91 for methyl *n*-butyl phthalate, 21.32–26.28 for DEHP and 16.61–23.02 for methyl 2-ethylhexyl phthalate. From these values, it is concluded that the phthalates interfere in the analysis of fatty methyl esters and other compounds.



Scheme I.

For example, among the seven columns studied, the DEGS column is often used for analysing fatty acid methyl esters. The ECL values of the phthalates and their transesterified products on the DEGS column were compared with those of the main unsaturated fatty acid methyl esters obtained from ref. 11. The ECL values of the methyl esters of oleic acid (18.51), linoleic acid (19.30), linolenic acid (20.40) and arachidonic acid (22.43) agreed very closely with those of methyl isopropyl phthalate, diisopropyl phthalate, methyl *n*-propyl phthalate and di-*n*-butyl phthalate, respectively. It is probable that these phthalates affect the quantitive and qualitative determination of these fatty acid methyl esters.

Using a DEGS column, Hasiak *et al.*[12] analysed the lipids of egg shell and shell membrane and found C19:1 and C21:0 methyl esters. These components com-

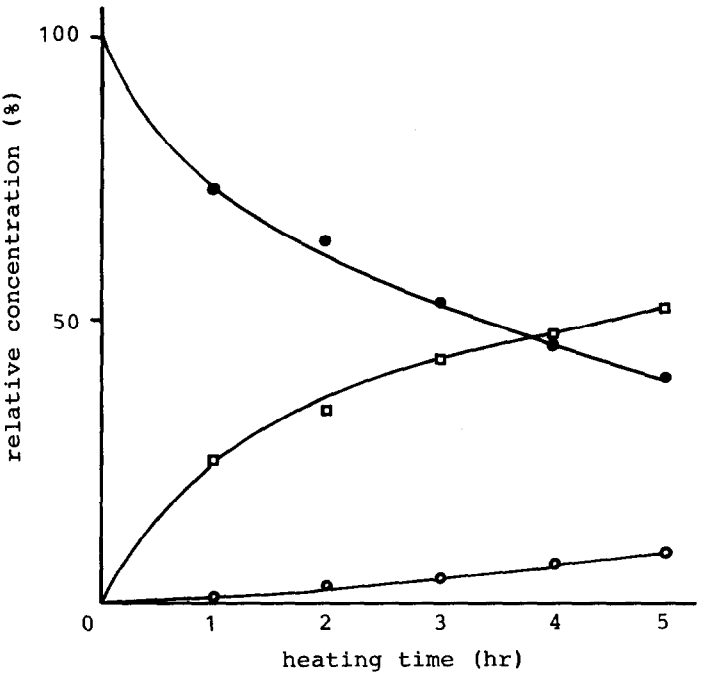


Fig. 2. Transesterification reaction of DBP at 90°C. ●, Di-*n*-butyl phthalate (DBP); □, methyl *n*-butyl phthalate; ○, dimethyl phthalate (DMP).

prised 15.4 and 41.9%, respectively, of all the methyl esters. However, Pascal and Ackman[13] concluded that these compounds were diisobutyl and di-*n*-butyl phthalate that had migrated from the equipment (vial caps). However, our results indicate that these compounds are transesterified phthalates, *i.e.*, methyl isobutyl phthalate and methyl *n*-butyl phthalate, respectively.

TABLE I

EFFECTS OF DIFFERENT CATALYSTS ON TRANSESTERIFICATION OF DBP AND DEHP

Transesterification conditions: heating at 80°C for 2 h.

Catalyst	*Relative concentration (%)*					
	DBP			*DEHP*		
	DBP	*Methyl n-butyl*	*DMP*	*DEHP*	*Methyl-2-ethylhexyl*	*DMP*
3% HCl–MeOH	58.12	37.69	4.19	61.45	38.55	trace
2% H_2SO_4–MeOH	70.62	27.72	1.66	81.37	18.63	–
2% BF_3–MeOH	84.75	14.84	0.41	88.58	11.42	–

TABLE II

ECL VALUES OF PHTHALATES AND THEIR MONOMETHYL ESTERS ON SEVEN COLUMNS

Phthalate	*Stationary phase**						
	DEGS	*SE-30*	*OV-17*	*SE-52*	*QF-1*	*PEG-20M*	*Apiezon M*
Dimethyl (DMP)	18.85	11.53	13.57	11.33	14.58	16.88	11.22
Diethyl	19.64	12.68	14.62	12.91	15.55	17.56	12.50
Methyl ethyl	–	12.08	14.07	12.24	15.10	17.27	11.84
Di-*n*-propyl	20.76	14.38	16.23	14.60	17.28	18.90	14.28
Methyl *n*-propyl	20.20	13.01	14.94	13.11	16.00	17.98	12.82
Diisopropyl	19.21	13.40	14.88	13.48	16.02	17.17	13.00
Methyl isopropyl	18.68	12.49	14.30	12.58	15.34	–	12.25
Di-*n*-butyl (DBP)	22.27	16.26	18.05	16.43	19.34	10.60	15.95
Methyl *n*-butyl	20.91	13.94	15.92	14.07	17.27	18.91	13.67
Diisobutyl	20.74	15.37	16.87	15.56	18.22	19.09	15.03
Methyl isobutyl	20.14	13.52	15.27	13.71	16.54	18.14	13.23
Di-*n*-amyl	23.86	18.10	19.88	18.31	21.16	22.31	17.80
Methyl *n*-amyl	21.77	14.89	16.90	15.08	18.20	19.82	14.68
Di-2-ethylhexyl (DEHP)	26.28	21.94	23.41	22.18	24.70	24.98	21.32
Methyl 2-ethylhexyl	23.02	16.99	18.78	17.24	20.16	21.36	16.61
Dinonyl	27.46	22.95	24.33	23.07	26.26	–	22.36
Methyl nonyl	23.48	17.50	19.28	17.58	20.80	–	17.10
Di-*n*-octyl	29.03	23.68	25.56	23.90	26.76	–	23.27
Methyl *n*-octyl	24.36	17.82	19.83	17.99	21.04	–	17.53
Dicyclohexyl	29.03	21.48	24.11	21.84	24.98	–	21.56
Methyl cyclohexyl	24.41	16.61	18.96	16.88	20.03	–	16.52
Butoxyethyl	29.33	21.08	23.54	21.51	–	–	20.43
Methyl butoxyethyl	24.61	16.44	18.82	16.80	–	–	16.02
Diphenyl	26.75	21.47	25.24	22.11	26.35	–	21.64
Methyl phenyl	25.01	16.65	19.59	16.97	20.75	–	16.62
Diallyl	21.97	14.16	16.29	14.46	17.10	19.59	13.89
Methyl allyl	20.64	12.95	14.97	13.09	16.00	18.49	12.61

* –, Undetectable.

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