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ANALYSIS OF FATTY ACID METHYL ESTERS ON AN SP 2340 GLASS CAPILLARY COLUMN

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SUMMARY

For the gas–liquid chromatographic separation of a complex mixture of fatty acid methyl esters, particularly the distribution of geometric isomers of unsaturated fatty acids, a glass capillary column coated with SP 2340 cyanopropylsiloxane stationary phase was prepared. Separations of 16:1ω 7c/16:1ω 7t, 22:1ω9c/22:1ω9t, of the group 18:1ω9t, 18:1ω7t, 18:1ω9c, 18:1ω7c, of all four geometric isomers of 18:2ω6 and other geometric and positional isomers of polyunsaturated fatty acids were achieved. The determination of the total serum fatty acids in healthy 10-year-old children and healthy 20-year-old adults was performed. In 35 min, over 100 peaks of serum fatty acids were detected. Increased levels of some saturated and monounsaturated fatty acids and decreased levels of linoleic acid and eicosadienoic acid (20:2ω6c,c) were found in the serum of adult subjects.

INTRODUCTION

Highly polar cyanopropylsiloxane stationary phases with good temperature stability show high selectivity in the chromatography of geometric and positional isomers of fatty acid methyl esters. The gas–liquid chromatographic (GLC) characteristics of these phases on packed columns have been described[1].

To prevent overlapping of some fatty acids in complex mixtures, more effective columns are necessary, such as capillary columns[2]. Preparation of capillary columns coated with cyanopropylsiloxane phases is, however, accompanied by technical problems, which leads to a decreased stability of these columns and their infrequent use in practice[3]. The purpose of our work was to prepare a glass capillary column coated with SP 2340 liquid stationary phase and to use it for the determination of fatty acids in the serum of 10-year-old children and 20-year-old adults.

EXPERIMENTAL

Chemicals

All individual standards of fatty acid methyl esters and standard mixtures were

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purchased from Supelco (Bellefonte, PA, U.S.A.). All were individually checked for purity by GLC under the same conditions used throughout the experiments and the final temperature was maintained long enough for free fatty acids and other components to be detected. All of the standards tested were more than 99% pure.

Analytical reagent grade solvents were used. Before the analysis they were redistilled and then purity was checked by GLC.

The blood donors were twenty healthy 10-year-old children and twenty healthy 20-year-old adults. The quantitative extraction of serum lipids was carried out by a modified Folch method as described by Nelson[4]. A 0.2-ml volume of serum was added to cold methanol, which contained as internal standards methyl heptadecanoate and methyl heneicosanoate. The total lipids were saponified with 1 *M* potassium hydroxide in ethanol at 80°C for 1 h in a nitrogen atmosphere. Free fatty acids resulted from hydrolysis with 4 *M* hydrochloric acid. The fatty acid methyl esters were prepared using diazomethane[5].

Chromatography

GLC was carried out on a Carlo Erba 2400 T gas chromatograph equipped with a flame-ionization detector. The chromatograph was fitted with a 78 m × 0.3 mm I.D. glass capillary column, coated with cyanopropylsiloxane SP 2340. The initial column temperature was 150°C, which was held for 3 min, then programmed from 150 to 220°C at 3°C/min. The maximum temperature was maintained for a further 9 min before cooling. The analysis time was 35 min. The injection and detector temperatures were 235°C. Hydrogen was used as the carrier gas with a linear velocity of 30 cm/sec. The splitting ratio was 50:1. The sample size was 1 μl (solutions in hexane). Peaks were identified by means of known standards and quantified with an SP 4000 Integrator (Spectra-Physics, Darmstadt, F.R.G.). Comparisons were made by Student's *t*-tests.

RESULTS AND DISCUSSION

Before coating, the capillary column was treated with water and then leached with 20% hydrochloric acid[6]. No other deactivation was used. The column was dynamically coated with SP 2340. The number of theoretical plates for stearic acid at 150°C was $N = 123,725$ (1586 per metre). Using *n*-hexadecanoic and *n*-heptadecanoic acid methyl esters, the separation number of the column was $TZ = 20.25$ (0.26 per metre). Although the specific separation number was much lower in comparison with that obtained by Jaeger *et al.*[7] with a capillary column coated with free fatty acid phase (FFAP), we achieved better separations of the isomers 16:1ω7c from 16:1ω7t (baseline), 18:1ω9t, 18:1ω9c, 18:1ω7c, and we achieved the separation of all four isomers of 18:2ω6 fatty acid (c = *cis*, t = *trans*). We also obtained a baseline separation of behenic and arachidonic acids and a partial separation of arachidonic and eicosatrienoic (20:3ω3c-all) acids as well. Earlier it was stated, that these three fatty acids eluted in the same peak[2]. The analysis time in this instance was three times shorter (Fig. 1). The stability of the column was about 400 analyses, during which period the change in retention times was less than 1%.

We tried to remove the slight tailing of peaks by deactivation of the surface with Carbowax 20M (Fig. 2) and by persilylation[6]. After deactivation with Carbowax

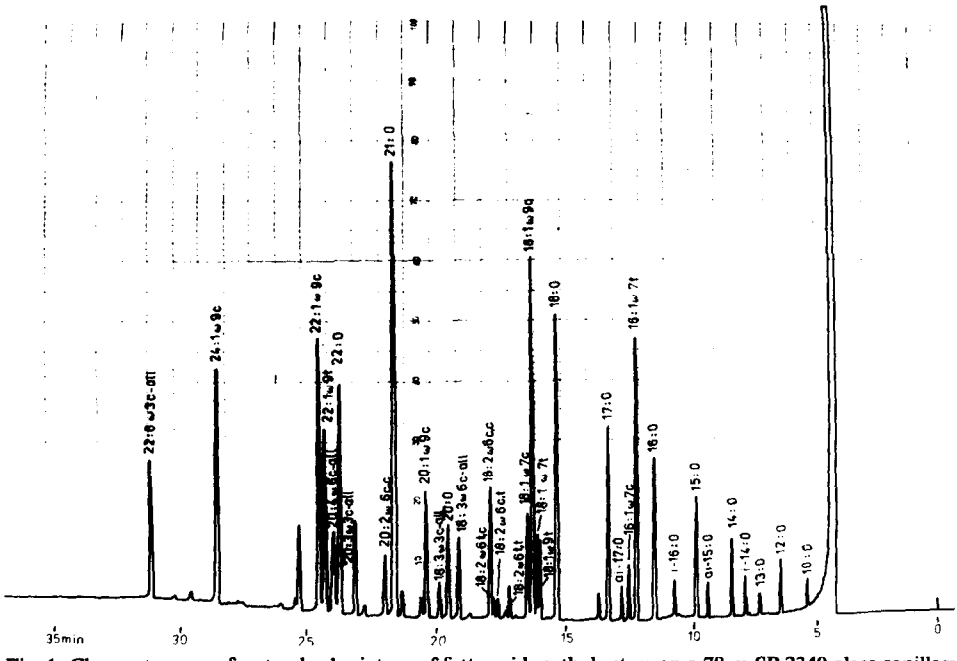

Fig. 1. Chromatogram of a standard mixture of fatty acid methyl esters on a 78-m SP 2340 glass capillary column. For operating conditions, see *Chromatography*.

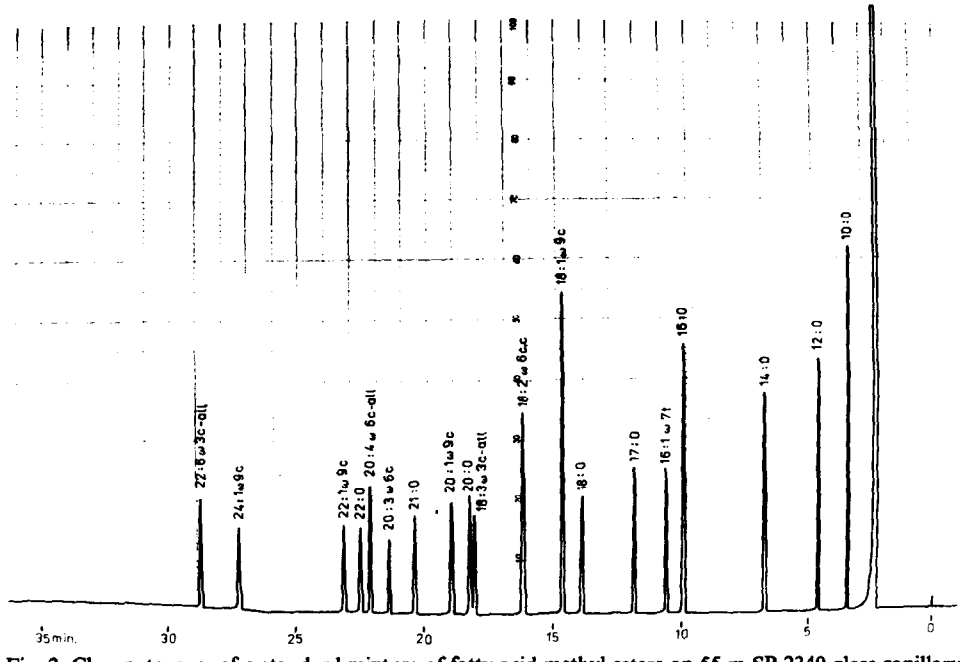

Fig. 2. Chromatogram of a standard mixture of fatty acid methyl esters on 55-m SP 2340 glass capillary column. Column temperature: 3 min initial hold, 140 to 220°C at 3°C/min., 9 min final hold at 220°C. Detector and injector temperature, 235°C. Carrier gas, hydrogen at a linear velocity of 41 cm/sec. Splitting ratio, 50:1.

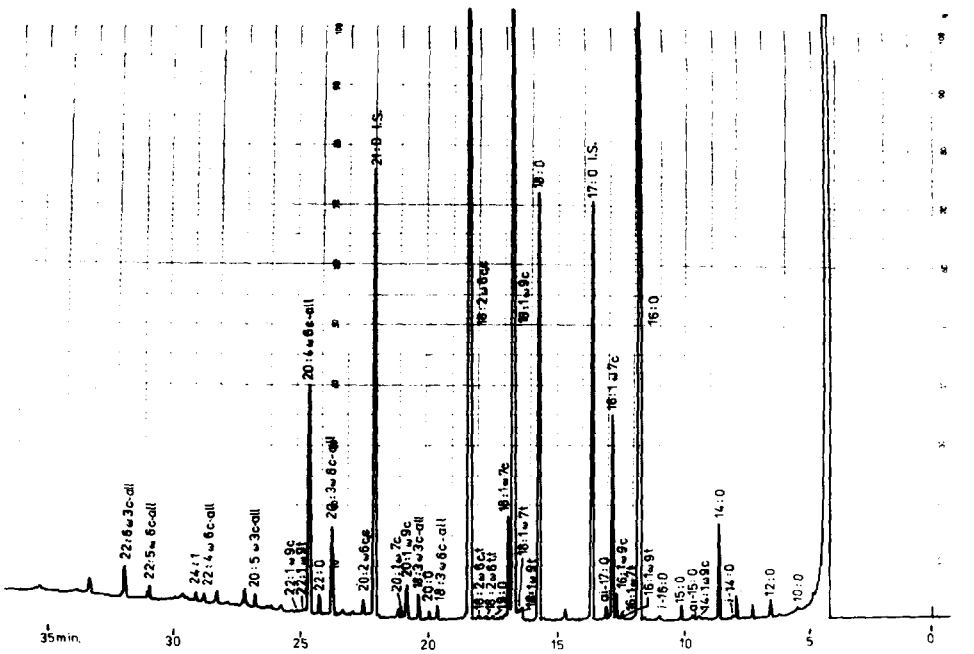

Fig. 3. Typical GLC pattern of human serum fatty acid methyl esters on a 78-m SP 2340 glass capillary column, including C17:0 and C21:0 as internal standards. For operating conditions, see *Chromatography*.

TABLE I

AVERAGE CALIBRATION FACTORS OF SOME FATTY ACID METHYL ESTERS (n = 7)

Fatty acid	*Calibration factor*	*Standard deviation*	*Relative standard deviation (%)*
10:0	74.05	4.20	5.7
12:0	68.48	3.42	5.0
14:0	62.33	3.56	5.7
16:0	50.39	1.22	2.4
16:1ω7c	56.86	1.16	2.0
18:0	40.21	0.52	1.3
18:1ω9c	43.76	1.25	2.9
18:2ω6c,c	44.67	0.44	1.0
18:3ω3c-all	55.85	1.89	3.4
20:0	38.97	0.36	0.9
20:1ω9c	45.05	0.50	1.1
20:3ω6c-all	45.42	0.87	1.9
20:4ω6c-all	46.15	0.72	1.6
22:0	39.21	0.30	0.8
22:1ω9c	44.12	0.46	1.0
24:1ω9c	43.69	1.24	2.8
22:6ω3c-all	53.57	0.41	0.8

TABLE II

FATTY ACID COMPOSITION OF TOTAL SERUM LIPIDS OF 10- AND 20-YEAR-OLD SUBJECTS

Values (%, w/w) are means ± standard deviations. ai = anteiso; i = iso.

Fatty acid	*10-year-old subjects*	*20-year-old subjects*
10:0	0.07 ± 0.02	0.07 ± 0.03
12:0	0.19 ± 0.10	0.41 ± 0.38*
14:0-i	0.13 ± 0.08	0.12 ± 0.08
14:0	0.76 ± 0.19	0.76 ± 0.19
14:1ω5c	0.12 ± 0.05	0.11 ± 0.04
15:0-ai	0.13 ± 0.05	0.12 ± 0.03
15:0	0.33 ± 0.06	0.31 ± 0.06
16:0-i	0.15 ± 0.06	0.18 ± 0.08
16:0-ai	0.06 ± 0.02	0.08 ± 0.04
16:0	17.83 ± 1.41	18.85 ± 1.37*
16:1ω9t	0.22 ± 0.08	0.32 ± 0.10***
16:1ω7t	0.06 ± 0.03	0.06 ± 0.04
16:1ω9c	0.63 ± 0.12	0.88 ± 0.20***
16:1ω7c	1.64 ± 0.37	1.88 ± 0.58
17:0-ai	0.26 ± 0.04	0.25 ± 0.06
17:0	Internal standard	
18:0	7.61 ± 0.60	6.94 ± 1.00*
18:1ω9t	0.81 ± 0.42	1.06 ± 0.80
18:1ω9c	19.88 ± 1.44	21.81 ± 2.26**
18:1ω7c	2.00 ± 0.35	2.27 ± 0.23**
19:0	0.07 ± 0.05	0.16 ± 0.08***
18:2ω6t,t	0.13 ± 0.07	0.14 ± 0.05
18:2ω6c,t	0.10 ± 0.04	0.13 ± 0.05
18:2ω6t,c	0.14 ± 0.07	0.29 ± 0.09***
18:2ω6c,c	33.05 ± 2.12	29.45 ± 3.24***
18:3ω6c-all	0.39 ± 0.16	0.57 ± 0.33
20:0	0.11 ± 0.05	0.12 ± 0.07
18:3ω3c-all	0.49 ± 0.11	0.49 ± 0.39
20:1ω9c	0.96 ± 0.55	0.85 ± 0.19
21:0	Internal standard	
20:2ω6c,c	0.52 ± 0.16	0.40 ± 0.09**
20:3ω6c-all	1.72 ± 0.33	1.70 ± 0.42
22:0	0.30 ± 0.14	0.30 ± 0.10
20:4ω6c-all	6.53 ± 2.22	6.45 ± 1.95
22:1ω9c	0.10 ± 0.07	0.14 ± 0.09
20:5ω3c-all	0.27 ± 0.14	0.28 ± 0.25
24:0	0.15 ± 0.07	0.16 ± 0.17
22:4ω6c-all	0.25 ± 0.11	0.35 ± 0.21
24:1ω9c	0.37 ± 0.20	0.31 ± 0.13
22:5ω6c-all	0.32 ± 0.15	0.40 ± 0.11
22:6ω3c-all	1.09 ± 0.53	0.95 ± 0.35

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

20M the tailing of peaks was removed, but the column had a lower specific separation number (TZ = 0.24 m) and lower stability (only about 200 analyses). The worst results were obtained on the column after persilylation. This column had a low efficiency and the peaks of fatty acid methyl esters were asymmetric.

A glass capillary column of length 78 m was used for the determination of serum fatty acids (Fig. 3). By using C17:0 as the internal standard fatty acids from the beginning of the chromatogram to C18:2 were quantified, whereas for the quantification of C18:3 and higher fatty acids C21:0 was used as the internal standard. Before the analysis of the samples calibration was performed. Table I gives average values of calibration factors, standard deviations and relative standard deviation for some fatty acid methyl esters, obtained from seven analyses of the test mixtures. The reproducibility of the chromatographic analyses expressed as relative standard deviation was about 1–3%; only for fatty acids with a small number of carbons (C_{10}–C_{14}) was it about 5%.

Table II shows serum fatty acid compositions of 10- and 20-year-old subjects. We found more than 100 different peaks. Only identified fatty acids are given. Identification of other peaks on chromatogram requires a combination of GLC with other methods (*e.g.*, mass spectrometry). The content of linoleate (18:2ω6c,c) is of special interest in view of reports[8,9] of the reduction of this fatty acid in patients with atherosclerosis and other diseases. Increased levels of saturated and monounsaturated fatty acids and decreased levels of essential fatty acids of serum in patients with hyperlipoproteinaemia were found[10].

We found significantly higher amounts of lauric acid, palmitic acid, oleic acid, *cis*-vaccenic acid and some other saturated and monounsaturated fatty acids in the serum of adults. On the other hand, in this group the levels of linoleic acid and eicosadienoic acid (20:2ω6c,c) were found to be lower. The results indicate that there are significant differences between the serum fatty acid compositions of 10-year-old children and 20-year-old adults, and may reflect uninterrupted atherogenesis.

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