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GAS CHROMATOGRAPHIC IDENTIFICATION OF FREE FATTY ACIDS IN TRACE AMOUNTS IN NATURAL MATERIALS

R. V. GOLOVNYA, M. B. TERENINA and V. P. URALETS
Institute of Organoelement Compounds, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)

SUMMARY

A method is proposed for the analysis of small amounts of complicated natural mixtures of fatty acids that are not chemically bonded in lipids. The analysis includes isolation of free fatty acids, formation of methyl esters, preparative gas chromatographic separation, hydrogenation of double bonds and analysis with a system of specially chosen columns.

The method was used successfully in the analysis of free fatty acids in salmon flesh in which 52 components were found and 43 were identified, consisting of odd- and even-carbon-numbered acids with 12–24 carbon atoms, 0–6 double bonds and also branched chains.

INTRODUCTION

A useful ancillary technique in the gas chromatographic analysis of fatty acid methyl esters (FAME) is hydrogenation of the double bonds of compounds leaving the gas chromatograph[1–4]. This technique requires relatively large amounts of the sample being investigated and is widely used in the fatty acid analysis of lipid glycerides. The study of free fatty acids (FFA) that are present in lipids in only small amounts, however, is difficult. Unfortunately, the known methods[5–9] combining hydrogenation and gas chromatographic separation, developed for model mixtures, have virtually no use, as the amount of sample necessary for reaction is too large and leads to overloading of the column and unsatisfactory chromatograms, due mainly to adsorption of FAME on the catalyst layer.

EXPERIMENTAL

The isolation of FFA from salmon flesh and the formation of methyl esters has been described earlier[10].

The preparative separation of the mixture of FFA methyl esters obtained was carried out with a Pye-Unicam 105 automatic preparative gas chromatograph on a glass column (210 × 0.95 cm) packed with 10% silicone OV-225 on Chromosorb W (45–60 mesh) at temperatures from 180 to 250° at a rate of 3°/min.

Analyses were carried out with a Pye-Unicam 104, Model 64, instrument

TABLE I

INFLUENCE OF SAMPLE SIZE ON THE RETENTION INDICES OF FATTY ACID METHYL ESTERS (FAME) IN A COLUMN PACKED WITH DEGS

FAME	*1% Pt on Chromosorb W (200°), 40-mm layer (120 mg)*					*1% Pt on Chromosorb W (200°), 6-mm layer (18 mg)*						
	Sample size (g)											
	2×10^{-6}	3×10^{-6}	5×10^{-6}	8×10^{-6}	2×10^{-5}	1×10^{-8}	1×10^{-7}	2×10^{-7}	1×10^{-6}	2×10^{-6}	1×10^{-5}	2×10^{-5}
18:0	2339	2314	2315	2314	2317	2313	2315	2314	2315	2314	2317	2323
19:0	–**	2408	2407	2409	2417	2407	2408	2408	2408	2407	2416	2420
20:0	–	2501	2502	2503	2514	2501	2500	2501	2501	2500	2512	2517
21:0	–	2603	2597	2595	2608	2594	2594	2595	2596	2596	2608	2615
22:0	–	–	–	2689	2705	2688	2688	2689	2690	2690	2705	2714
18:2						2314	2314	2316	2313	2317	2314	2346
18:3						2314	2314	2313	2312	2316	2315	2316
20:4						2502*	2501	2501	2500	2505	2504	2524

* Sample size 5×10^{-8} g.

** In these conditions the acid was adsorbed.

with a dual flame ionization detector and glass columns (150 × 0.4 cm) with 5% DEGS, silicone OV-225 on Chromosorb W (100–120 mesh) and 3% silicone SE-30 on Gas-Chrom Q (80–100 mesh). The temperature of analysis was 180° in the first column and 200° in the others. Retention data are presented as Kováts indices.

The hydrogenation catalyst was prepared by using a modified method[6]. Chromosorb W (2.5 g) was added to an aqueous solution of platinum chloride (0.04 g). After stirring, water was removed with a film evaporator. The catalyst was dried at 110° for 5 h and activated in a stream of hydrogen for 1 h at 170° and then for 1 h at 200°.

The catalyst was placed at the beginning of the chromatographic column between two pieces of glass-wool. The amounts of the catalyst used were 120 or 18 mg (40- or 6-mm layer). The other parallel column contained, instead of catalyst, an equal amount of pure Chromosorb W. The carrier gas in the first column was hydrogen and in the second helium (flow-rate, 40–70 ml/min).

The standard substances were saturated C_{14}, C_{16}, C_{18}–C_{22} FAME, oleic, linoleic, linolenic and arachidonic acid methyl esters, and their ethereal solutions were used for analysis.

RESULTS AND DISCUSSION

We established hydrogenation conditions that enable the catalyst adsorbtion activity and correspondingly the amount of analyzed sample to be reduced considerably, by choosing the best catalyst support, the optimal amount of catalyst and the best reaction temperature (200°). We used Chromosorb W as a catalyst support, which, according to a study of Krupčik *et al.*[11], does not adsorb FAME, unlike Chromosorb P and the other supports used earlier[5–9].

The results of experiments with a standard mixture of FAME are presented in Table I. When the amount of catalyst used is 120 mg (40-mm layer) (usually recommended), we obtained the results given in the left-hand part of Table I. The complete disappearance of FAME peaks owing to adsorption was observed when amounts of sample of less then 2–6 × 10^{-6} g were injected. The adsorption increases with increase in the molecular weight of the investigated FAME. A 10-fold increase in sample size (2 × 10^{-5} g) leads to column overloading, which can be seen by an abnormal change in the retention indices and distorted peaks.

A decrease in the amount of catalyst to 18 mg (6-mm layer) enables the sample size to be reduced considerably (100 times). The retention indices remain virtually constant over a large interval (1 × 10^{-8}–2 × 10^{-6} g), which makes it possible to analyse natural mixtures with different concentrations of components. The amount injected must not exceed 1 × 10^{-5} g, this being the limit of the column capacity.

Analyses were carried out with a system of columns having different polarities. Seven stationary phases recommended in the literature were tested, and three of them were chosen for analysis. Retention indices have been used for the first time to characterize the gas chromatographic behaviour of high-boiling FAME. We have determined a linear dependence between the number of carbon atoms (n) and retention indices of standard saturated FAME: $I_{200}^{SE} = 100n + 314$; $I_{200}^{OV} = 102n + 517$;

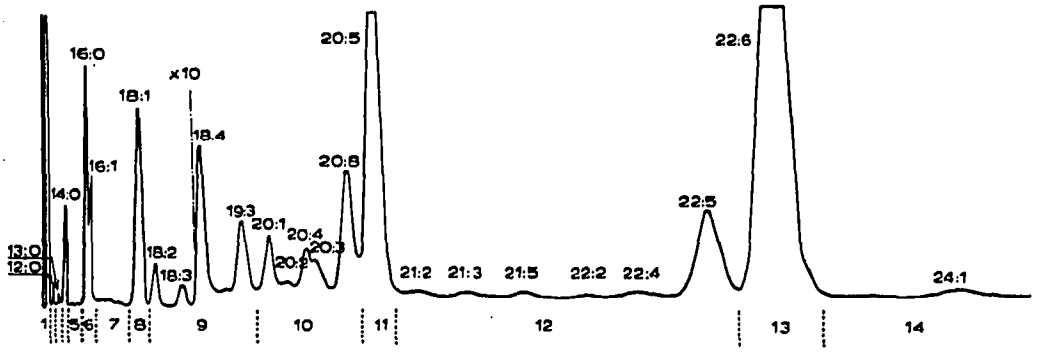

Fig. 1. A chromatogram of a mixture of free fatty acid methyl esters isolated from salmon flesh in the column packed with 5% DEGS on Chromosorb W at 200°. Under the base-line are shown preparatively separated fractions.

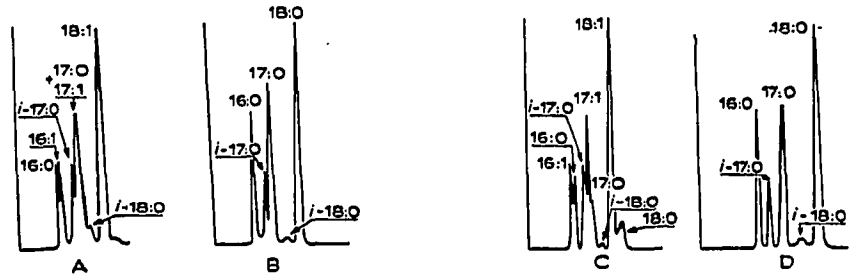

Fig. 2. Chromatograms of the seventh fraction on columns packed with 5% DEGS on Chromosorb W (A and B) and 3% SE-30 on Gas-Chrom Q (C and D) with (B and D) and without (A and C) a hydrogenation catalyst.

$I_{180}^{DEGS} = 94n + 621$. Unsaturated FAME have the following retention indices: $C_{18:1}$, $I_{200}^{SE} = 2085$, $I_{200}^{OV} = 2367$ and $I_{180}^{DEGS} = 2326$; $C_{18:2}$, $I_{200}^{SE} = 2085$, $I_{200}^{OV} = 2400$ and $I_{180}^{DEGS} = 2363$; $C_{18:3}$, $I_{200}^{SE} = 2085$, $I_{200}^{OV} = 2442$ and $I_{180}^{DEGS} = 2416$; $C_{20:4}$, $I_{200}^{SE} = 2235$, $I_{200}^{OV} = 2617$ and $I_{180}^{DEGS} = 2576$. The difference in the retention indices on columns packed with OV-225 and SE-30 (ΔI_{200}^{OV-SE}) was found to be dependent on the number of double bonds in the analyzed FAME and can be characteristic of the degree of unsaturation.

One of the chromatograms of the investigated mixture of FFA methyl esters isolated from salmon flesh is presented in Fig. 1. Fourteen fractions collected with the preparative instrument are indicated under the base-line. The analytical chromatograms of the seventh fraction are shown in Fig. 2 as an example. By comparing chromatograms before and after hydrogenation and calculating the retention indices, it is easy to identify compounds, as indicated in Fig. 2. All separated fractions were examined this way. Some compounds that were specially purified chromatographically were analyzed by mass spectrometry. The results obtained confirmed the results of the gas chromatographic identification. Nuclear magnetic resonance and mass spectrometric analysis of the component with 22 carbon atoms in fraction 13 revealed the number and sequence of double bonds: 4, 7, 10, 13, 16, 19.

TABLE II
FREE FATTY ACIDS FOUND IN SALMON FLESH

Methyl ester	*Relative ratio (%)*	*Methyl ester*	*Relative ratio (%)*
12:0	0.15	20:0	Trace
13:0	0.08	20:1*	Trace
14:0	3.8	20:2*	0.08
14:1*	0.5	20:3*	2.4
iso-15:0	0.26	20:4	0.7
15:0	0.37	20:5	7.8
iso-16:0	Trace	21:0	Trace
16:0	18.0	21:2*	0.06
16:1	9.1	21:3*	0.07
iso-17:0	0.3	21:5*	0.16
17:0	0.17	22:2*	0.14
17:1	0.6	22:4*	0.11
iso-18:0	Trace	22:5	3.6
18:0	1.6	22:6	12.1
18:1	25.9	23:2*	Trace
18:2	6.3	24:0	Trace
18:3	1.2	24:1	0.2
18:4	0.4	23:5*	Trace
19:0	0.04	24:3*	Trace
19:2*	0.05	24:4*	Trace
19:3*	0.2	24:5*	Trace
iso-20:0*	Trace		

* Tentative identification.

The components of free fatty acids identified in salmon flesh are listed in Table II. As expected, the amount of even-carbon-numbered FFA predominates over odd-carbon-numbered FFA (4% relative). It is interesting that branched-chain FFA were found (<1% relative), which were not detected earlier[12,13].

Hence, free fatty acids in fish have been studied for the first time. In spite of the fact that the analysis of free fatty acids is more complicated than that of the fatty acids of triglycerides, the number of compounds identified in this study exceeds those found earlier[12,13] in products of triglyceride hydrolysis.

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