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Note

Gas—liquid chromatographic analysis of intact long-chain triglycerides

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Direct gas—liquid chromatographic (GLC) analysis of triglycerides was first described more than 20 years ago [1—3]. Kuksis and co-workers [4—6] and Litchfield et al. [7] have made great advances in the theory and practical applications of this method. Triglycerides with carbon numbers of over 60 have been analysed by Litchfield et al. [8] and also by other authors [9, 10]. Quantitative analysis of low quantities of these compounds is very difficult because of their low recovery. In natural materials, triglycerides with higher carbon numbers appear primarily in the fat of sea animals. These triglycerides contain highly unsaturated long-chain fatty acids. This fact is the cause of their chemical heterogeneity resulting in poor GLC separation. Therefore, samples are often hydrogenated prior to analysis [8]. The hydrogenation is not difficult, but it represents several analytical operations that are not desirable in the analysis of large series of samples with low contents of triglycerides. The great number of analytical operations also has a negative effect on the reproducibility of the analysis. A further problem of the quantitative analysis of triglycerides with higher carbon numbers is commercial unavailability of these compounds in the pure form. Direct determination of the correction factors is not possible and the literature data cannot be generally used [11].

This paper describes optimization of the analytical conditions for the analysis of low amounts of long-chain triglycerides in blood plasma. Optimal conditions were defined by lowest possible values of f_w (weight correction factor; the ratio of weight percentage to area percentage for the individual compounds) and ΔC (peak resolution, which represents minimal carbon number difference between two compounds with baseline separation). Using the optimized method, which does not require sample hydrogenation, an effect of dietary mackerel fat on the plasma triglyceride composition was studied.

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MATERIALS AND METHODS

Materials

Triarachidin and tristearin were obtained from Supelco (Bellefonte, PA, U.S.A.). The Gas-Chrom Q support, 100—120 and 120—140 mesh, and the OV-1 stationary phase were supplied by Applied Science Labs. (State College, PA, U.S.A.) and Serva Feinbiochemica (Heidelberg, G.F.R.). The Supelcoport 100—120 mesh and the Chromosorb 750 100—120 mesh supports were supplied by Supelco. All solvents used were of analytical grade, supplied by Lachema (Brno, Czechoslovakia) and they were distilled before use.

Methods

The plot of f_w vs. the amount of injected compound was measured using the temperature programme, 5 or 8°C/min, and the optimized carrier-gas flow-rate (individually for each column). Before the measurement, recovery of the test compounds was stabilized by five injections of 5 μg of triarachidin. The plot of f_w vs. the carrier-gas flow-rate was measured using an injected amount of 300 ng of triarachidin and the temperature programme, 5 or 8°C/min. Analogously, the plot of f_w vs. the temperature programme rate was measured

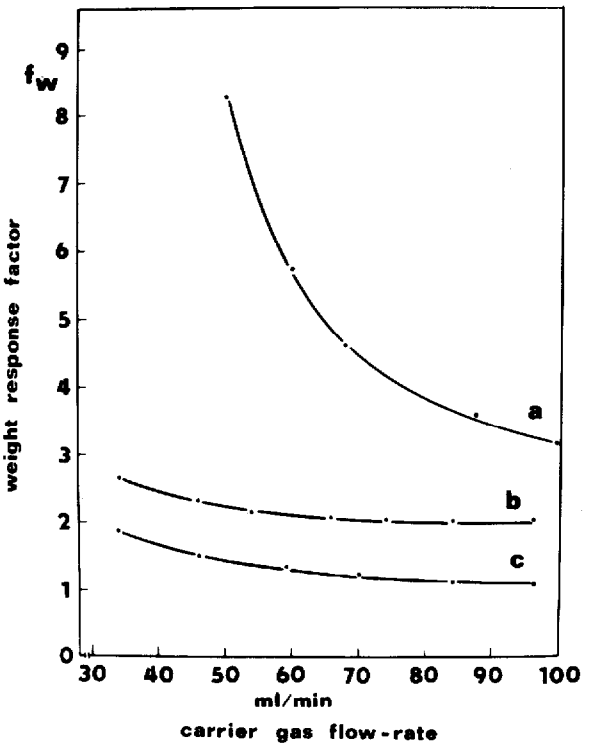

Fig. 1. Plot of f_w vs. the carrier-gas flow-rate for triarachidin measured with different columns at the same temperature programme rate (8°C/min) and the same analyzed amount of triarachidin (300 ng). (a) 3% OV-1 on Chromosorb 750, 100—120 mesh; (b) 1.5% OV-1 on Gas-Chrom Q, 120—140 mesh; (c) 1.5% OV-1 on Supelcoport, 100—120 mesh. For other analytical conditions see under Methods.

using the optimized carrier-gas flow-rate — concerning the peak resolution (ΔC) [7] and f_w values — for each column. Under the optimized conditions, the plots of ΔC vs. the carrier-gas flow-rate and the temperature programme rate were also measured. The samples contained the same amounts by weight of tristearin and triarachidin.

Apparatus and operating conditions

The analyses were performed on Perkin-Elmer gas chromatographs F-30, F-17 and 900. Peak areas were integrated using a Perkin-Elmer M-2 calculating integrator. In all cases, glass columns 0.5 m × 2.0 mm I.D. were used. The F-30 was equipped with on-column injection; the other gas chromatographs were equipped with the usual glass-lined injectors. Column packings were prepared in our laboratory by an evaporation technique. The columns were packed using the combined effect of suction and vibration.

Analytical conditions. Injector temperatures 300°C (F-30 and 900) and 320°C (F-17), detector temperatures 350°C (F-30 and 900) and 320°C (F-17), oven temperature programme 180—350°C, 2—10°C/min. Helium carrier gas was used at flow-rates of 30—180 ml/min. Quantitative analysis was performed using an internal standard method.

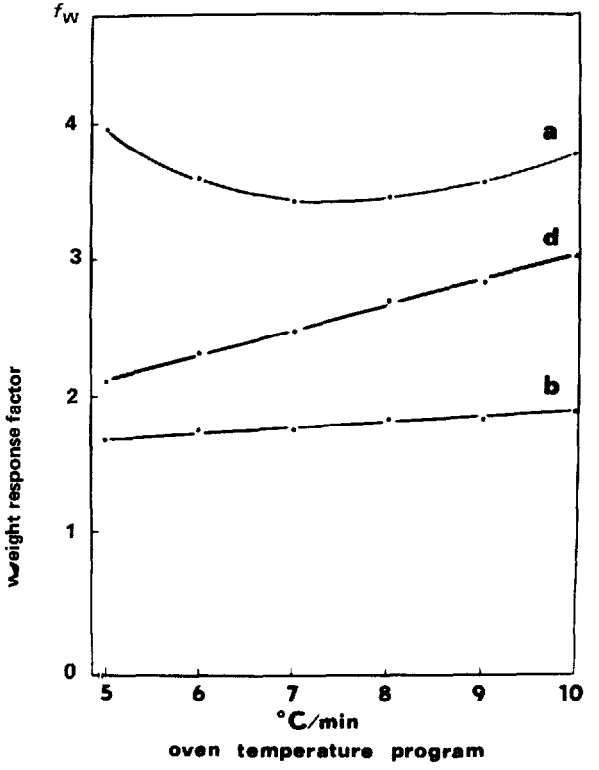

Fig. 2. Plot of f_w vs. the temperature programme rate for triarachidin measured with different columns at optimal carrier-gas flow-rates. (a) 3% OV-1 on Chromosorb 750, 100—120 mesh, carrier-gas flow-rate 90 ml/min; (b) 1.5% OV-1 on Gas-Chrom Q, 120—140 mesh, carrier-gas flow-rate 60 ml/min; (d) 1% OV-1 on Gas-Chrom Q, 100—120 mesh, carrier-gas flow-rate 44 ml/min. For other analytical conditions see under Methods.

RESULTS

First, the effect of different supports and loading with stationary phase on the recovery of the model compound, triarachidin, was studied. The f_w value was the criterion of the recovery of the compounds analysed; the values of the weight correction factors for 315, 100 and 56 ng of triarachidin are given in Table I. These values are also dependent on other parameters, especially on the carrier-gas flow-rate and the temperature programme rate. The course of these plots for some of the columns is given in Figs. 1 and 2. In addition to the recovery of triglycerides with high carbon numbers, the column should also attain the highest possible peak resolution. This value is again dependent on the above parameters. Our results obtained with the selected columns are shown in Figs. 3 and 4. Some practical applications of the results of the optimization in the analysis of neutral blood plasma lipids are shown in Fig. 5.

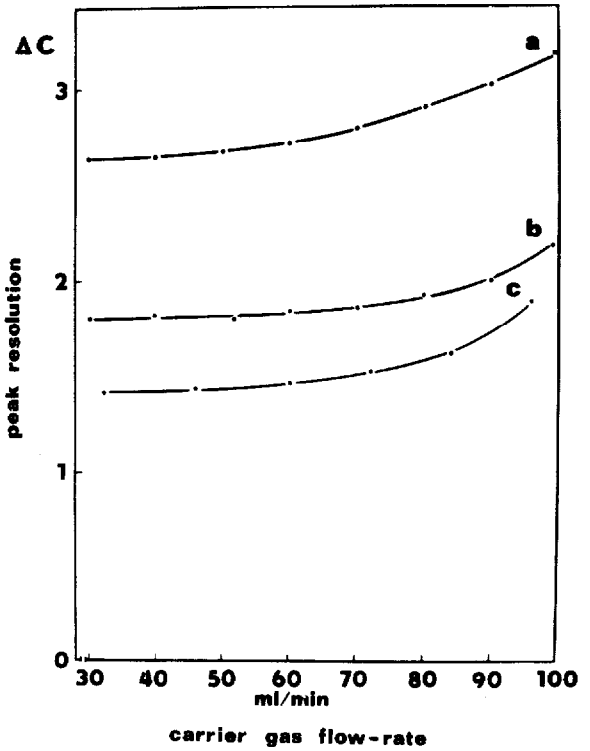

Fig. 3. Plot of ΔC vs. the carrier-gas flow-rate for triglycerides C_{54}—C_{60} using different columns. Analytical columns are the same as in Fig. 1.

DISCUSSION

The course of the plot of f_w vs. the injected amount of triarachidin is different for each individual instrument, even if the same column packing is used, as is shown in Table I. This observation is in good agreement with the results published by Litchfield et al. [7]. The course of the plot mentioned above and the f_w values are similar when they are measured with a series of

TABLE I

THE f_w VALUES FOR TRIARACHIDIN MEASURED WITH DIFFERENT COLUMNS UNDER OPTIMIZED CONDITIONS

Column	Instrument	Packing	Carrier-gas flow-rate (ml/min)	Oven temperature programme rate (°C/min)	f_w*		
					315 ng	100 ng	56 ng
A-33	F-30	1% OV-1 on Gas-Chrom Q, 100—120 mesh, batch No. 749	67	5	1.97	3.10	4.20
A-43	F-30	1% OV-1 on Gas-Chrom Q, 100—120 mesh, batch No. 749	75	5	2.02	3.10	4.10
A-51	F-17	1% OV-1 on Gas-Chrom Q, 100—120 mesh, batch No. 749	72	5	2.04	5.67	>10
B-79	F-30	1.5% OV-1 on Gas-Chrom Q, 120—140 mesh, batch No. 1422	60	8	2.74	6.77	9.38
A-74	F-30	3% OV-1 on Chromosorb 750, 100—120 mesh, batch No. 102	90	8	2.43	5.18	9.95
B-69	F-30	1% OV-1 on Gas-Chrom Q, 100—120 mesh, (Serva, batch No. 46559)	44	8	2.16	3.10	4.65
A-48	900	1% OV-1 on Gas-Chrom Q, 100—120 mesh, batch No. 749	77	5	3.16	5.94	9.43

*Each value represents the mean of two measurements. Values of f_w were measured with a C.V. better than 5% (calculated from 50 measurements).

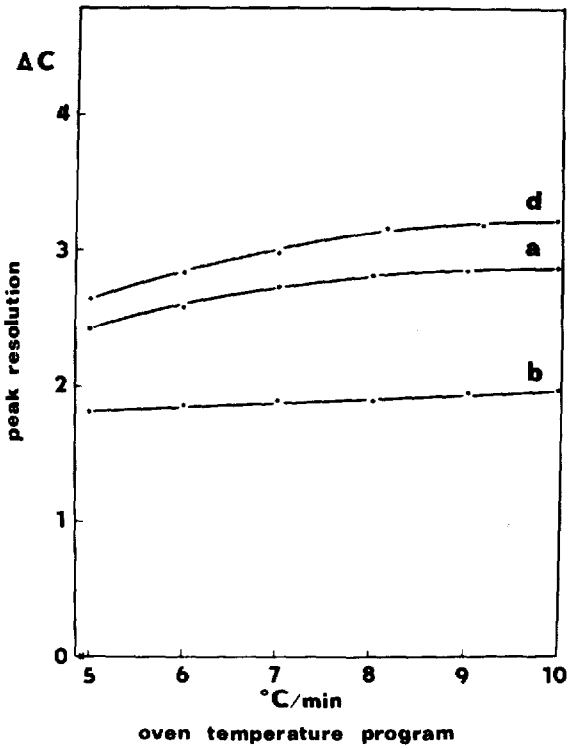

Fig. 4. Plot of ΔC vs. the temperature programme rate measured with the different columns. Analytical conditions are the same as in Fig. 2.

columns of the same length and packing, using the same instrument. Different f_w values were measured for the same analysed amount of triarachidin when different supports and different loading with stationary phase were used.

The f_w values for the given column and instrument are also dependent on the carrier-gas flow-rate and the temperature programme rate. These results are also in agreement with those published by Litchfield et al. [7] for the region of higher concentrations, where the f_w values are independent of the injected amount, and for triglycerides with lower carbon numbers.

The course of the plot of f_w vs. the carrier-gas flow-rate was the same for all the columns tested. The column packed with 3% OV-1 on Chromosorb 750 yielded high f_w values for low carrier-gas flow rates. On the other hand, the columns with lower loading of the support with the stationary phase yielded a sufficient recovery of triarachidin using relatively low carrier-gas flow rates.

The plot of f_w vs. the temperature programme rate was different for the different columns. The columns with the lower loading of the support with the stationary phase yielded a linear plot of f_w vs. programme rate. The same plot was non-linear using columns packed with 3% of OV-1 on Chromosorb 750. So far, we do not have a satisfactory explanation for this effect, which was observed repeatedly.

The higher values of the carrier-gas flow-rate, which are necessary for adequate f_w values (measured using the columns with 3% OV-1) result in decreased peak resolution. From this point of view, it is more advantageous to

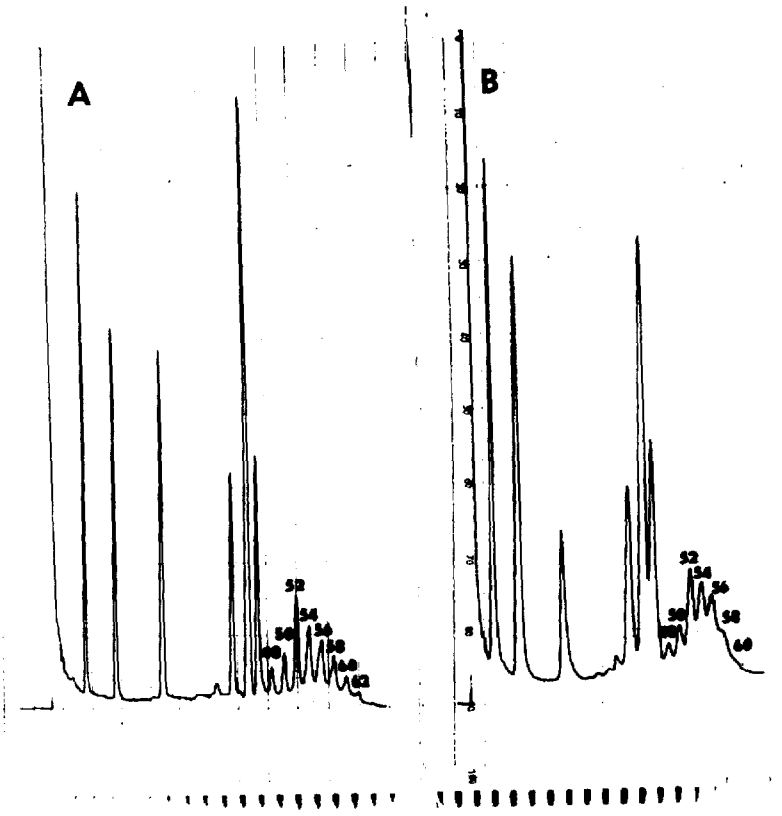

Fig. 5. (A) Gas chromatogram of plasma neutral lipids after the experimental diet containing the mackerel fat. Column was packed with 1.5% OV-1 on Gas-Chrom Q, 120—140 mesh. (B) Gas chromatogram of the same sample as in Fig. 5A. Column was packed with 3% OV-1 on Chromosorb 750, 100—120 mesh. For analytical conditions see under Methods and Table I. The figures 48—62 correspond to the carbon numbers of individual triglyceride fractions (carbon number is defined as the sum of the carbon atoms of the individual fatty acid moieties).

use the columns with a lower loading of the support with the stationary phase. Such columns yield markedly lower f_w values using 2—3 times lower flow-rates.

The peak resolution dependence on the temperature programme is similar for the different columns. It is probable that neither the ΔC value nor the course of the plot are basically different for the different loading of the support with the stationary phase. The high peak resolution of the columns with low loading can be illustrated by the results of the analysis of a sample of blood neutral lipids, which contains markedly heterogeneous triglycerides. The weight limit for the quantitative analysis of triglycerides with carbon number of over 60 is about 50 ng.

CONCLUSIONS

Quantitative analysis of higher molecular weight triglycerides in the concentration region where the f_w values are dependent on the amount

analyzed, requires individual optimization of the parameters for each column. Columns with a lower loading of support with the stationary phase yield more favourable values for the weight correction factors using relatively low carrier-gas flow-rates, and their peak resolution is good. The exact explanation of the role of the support under limit conditions of gas chromatography is very complicated and requires further experiments.

Comparing the supports under study, very good results were obtained using Gas-Chrom Q and Supelcoport, but significant differences were observed between individual batches of the same support. The quality of the column is also influenced by the coating technique of the support with the stationary phase, as well as by the technique of column packing and other factors already described [7, 9].

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