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Analysis of human blood plasma triacylglycerols using capillary gas chromatography, silver ion thin-layer chromatographic fractionation and desorption chemical ionization mass spectrometry

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ABSTRACT

The identification and quantitation from pooled human plasma of over 50 molecular species of triacylglycerols, including 22 not previously reported, are reported. The triacylglycerols were first resolved by silver ion thin-layer chromatography into seven fractions, which were independently analysed by polarizable capillary gas chromatography and desorption chemical ionization mass spectrometry in the presence of an internal standard. The two methods gave similar values for the estimates of oligoenoic species, but the former method underestimated the polyenes (due mainly to significant losses caused by thermal degradation), and the latter method overestimated the saturates. The results show that an effective analysis of molecular species of plasma triacylglycerols cannot be obtained by either technique alone.

INTRODUCTION

The use of a capillary column with a polarizable stationary phase [1] made it possible to analyse triacylglycerols (TGs) in natural materials in detail. This type of stationary phase makes it possible to separate TGs according to the number of double bonds and the carbon number. Such columns have been used to study the composition of plant and animal lipids [2–5], but not previously for the analysis of blood lipids. Only two chromatograms of human blood TG lipids obtained with the aid of a polarizable capillary column have been published [6,7], and the information obtained was much more detailed than when non-polar capillary

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columns or even packed columns were used. However, problems occurred in the identification of individual peaks, but this shortcoming was overcome by means of gas chromatography–mass spectrometry (GC–MS) [8]. Combination of high-performance liquid chromatography (HPLC) with mass spectrometry has been used [9] but the results were incomplete. A non-polar column and GC–MS identification combined with stereospecific analysis have also been used [10,11].

This paper describes the analysis of human blood TGs using two independent techniques, capillary gas chromatography (GC) after silver ion thin-layer chromatography (TLC) and desorption chemical ionization mass spectrometry (DCI-MS).

EXPERIMENTAL

Chemicals

TGs (30:0, 36:0, 42:0, 54:0, 54:3, 54:6, 54:9, 57:0 and 60:0) and fatty acid methyl esters (FAMEs) (14:0, 16:0, 16:1, 18:0, 18:2, α-18:3, Γ-18:3, 20:0, 20:3, 20:4, 20:5, 22:0, 22:6 and 24:0) were obtained from Sigma (St. Louis, MO, USA). Analytical-grade solvents were from Lachema (Brno, Czechoslovakia). Silica gel $HF_{254+366}$ were supplied by Merck (Darmstadt, Germany).

Human blood plasma

Ten samples containing 2 ml of fresh human blood plasma (from fasting subjects) were mixed, and the total lipids were extracted [12]. TGs were separated by silver ion TLC [13].

Capillary GC of intact TGs

Total TGs and TGs fractions after silver ion TLC were analysed on a fused-silica capillary column (25 m × 0.25 mm I.D. containing 0.1 μm TAP phase) (Chrompack, Middelburg, Netherlands). The carrier gas was hydrogen (100 cm/s). The temperature was programmed from 320 to 350°C at 1.5°C/min. The OCI-3 injector (SGE, Kensington, Australia) was adapted to the "moveable on-column" injection technique [14,15]. The injection temperature was 120°C. A flame ionization detector was used at 375°C, and a chromatographic apparatus PU 4900 (Pye Unicam, Cambridge, UK) was used for the analysis. The samples were dissolved in isooctane, and 0.2 μl of a 0.1% solution was injected (57:0 was the internal standard).

Desorption chemical ionization MS

A Finnigan MAT (Finnigan, Germany) mass spectrometer was used. The ion-source temperature was 200°C, the heating rate 1°C/s, and the reaction gas ammonia ($1.3 \cdot 10^{-4}$ bar).

FAME and TG fractions

TG fractions were reesterified [16] and analysed on a capillary column (10 m × 0.25 mm I.D., 0.2 μm film thickness with CP-Wax 52, *i.e.* chemically bonded Carbowax 20 M) (Chrompack). A PU 3900 gas chromatograph was used. Hydrogen was used as the carrier gas (60 cm/s), and the column temperature was programmed from 165 to 235°C at 5°C/min. The individual FAMEs were identified on the basis of comparison of their retention data with the retention data of standards [17].

RESULTS AND DISCUSSION

Fig. 1 shows a gas chromatogram of total human blood TGs. Part of the mass spectrum of the sample covering the range of pseudomolecular ions (*m/z* 800–940) is shown in Fig. 2. Individual TGs were quantified on the basis of the abundance of the pseudomolecular ions.

A gas chromatogram of the FAMEs of polyene fraction No. 7 is shown in Fig. 3, and the corresponding DCI mass spectrum in Fig. 4. Pseudomolecular ions with *m/z* 927, 925 and 923, corresponding to TGs 56:5, 56:6 and 56:7, were very well discriminated. Table II lists all the TGs of human plasma that have already

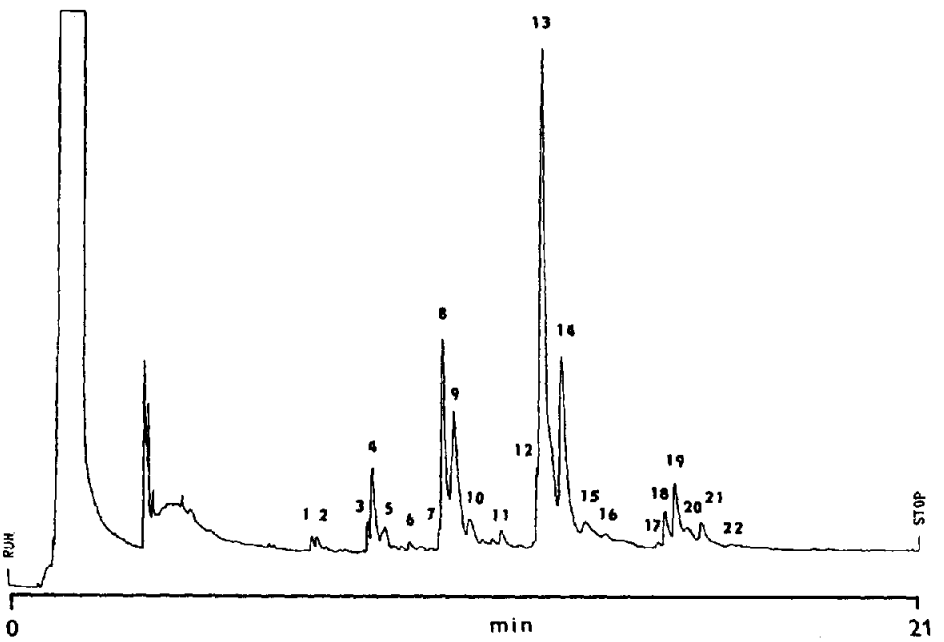

Fig. 1. Capillary gas chromatogram of human blood plasma total TGs. Peaks: 1 = MPP; 2 = MMO + MPPo; 3 = PPP; 4 = PPPo; 5 = MPoO + MPL; 6 = PdPoS; 7 = PPS; 8 = PPO; 9 = PPoO + PPL; 10 = PPoL; 11 = PdOS; 12 = PSO; 13 = POO; 14 = POL; 15 = PLL + PoOL; 16 = HdOO; 17 = SSO; 18 = SOO; 19 = OOO; 20 = SOL; 21 = OOL; 22 = OLL. For fatty acid abbreviations see Table II. Analytical conditions as in Experimental.

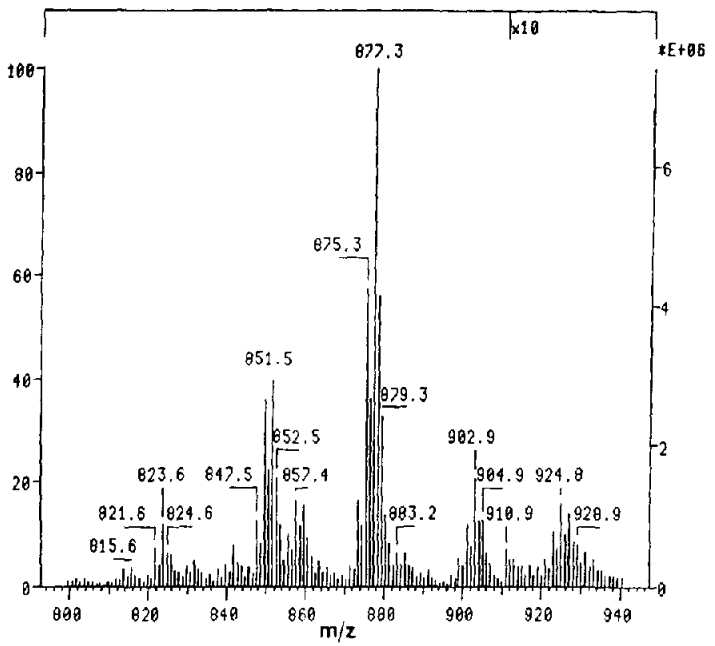

Fig. 2. DCI mass spectrum of human blood plasma total TGs. Conditions as in Experimental.

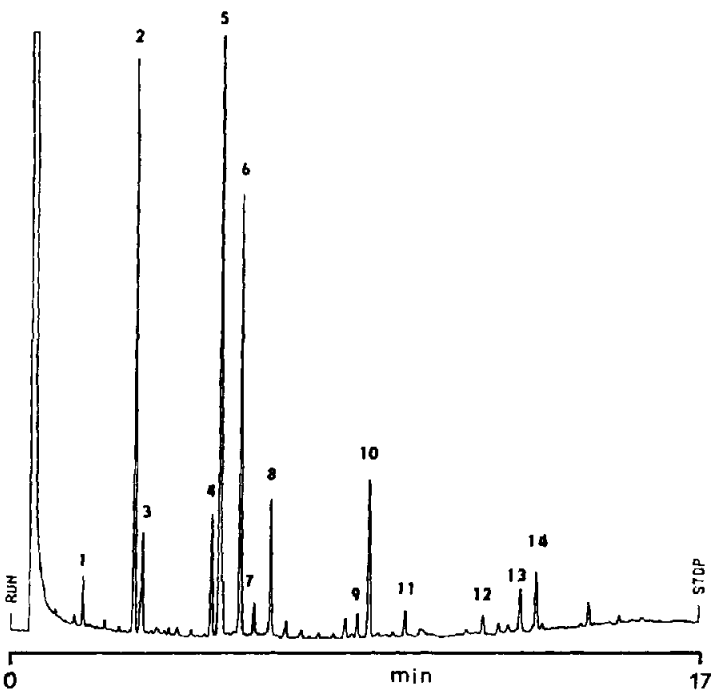

Fig. 3. Capillary gas chromatogram of fatty acids of polyenoic fraction of human blood plasma TGs (fraction 5/122+, see Table I). Peaks: 1 = 14:0; 2 = 16:0; 3 = 16:1; 4 = 18:0; 5 = 18:1; 6 = 18:2; 7 = 18:3*n*-6; 8 = 18:3*n*-3; 9 = 20:3*n*-6; 10 = 20:4*n*-6; 11 = 20:5*n*-3; 12 = 22:4*n*-6; 13 = 22:5*n*-3; 14 = 22:6*n*-3. Analytical conditions as in Experimental.

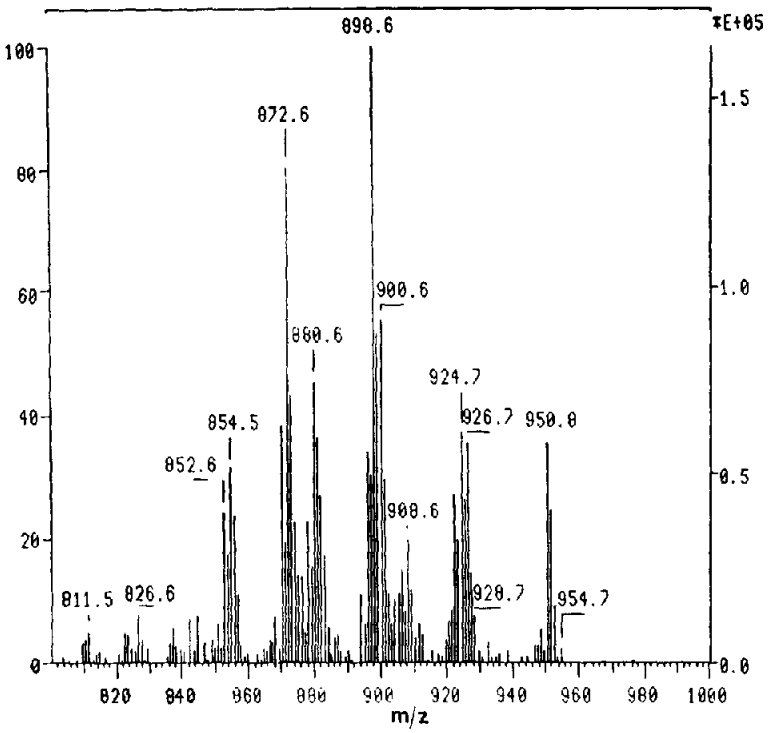

Fig. 4. DCI mass spectrum of polyenoic fraction of human blood plasma TG (fraction 5/122+, see Table I). Conditions as in Experimental.

been described (bold letters) and those discovered in this investigation by means of CGC and DCI-MS, either in the initial sample or in silver ion TLC fractions. The discovery of previously unreported TGs in human plasma does not contradict literature data [6,7], because two independent techniques were used (silver ion TLC with capillary and DCI-MS).

The comparison of quantitative data obtained by capillary GC and DCI-MS is also shown in Table II. The values are presented either as molar percentages (capillary GC) or as relative abundances of corresponding pseudomolecular ions (DCI-MS). The primary identification was performed on the basis of the relationship between structure and elution temperature [3]. TGs identified in these preliminary experiments were verified according to the pseudomolecular ions in DCI-MS of the initial sample or in fractions after silver ion TLC. Only fraction 7 (polyene TG) is exceptional. The finding of the TGs with summary formulae 56:5, 56:6 and 56:7 in this fraction was better on the basis of DCI-MS than by capillary GC. This assumption was also verified experimentally by analysis of a model sample of TGs with known proportions of TGs 54:0, 54:3, 54:6 and 54:9. However, it appears from DCI-MS that the composition of TGs in this fraction is

TABLE I

FATTY ACID COMPOSITION (mol %) OF INDIVIDUAL TG FRACTIONS

Fatty acid[a]	Silver ion TLC fraction / TG class[b]						
	0 / 000	1 / 001	2 / 011	3[c] / 111 + 002	3 / 012	4 / 112 + 022	5 / 122 + other[d]
14:0	9.81	5.36	2.42	1.79	1.69	1.15	0.73
15:0	1.54	0.67	0.37	0.29	0.32	0.20	0.32
16:0	64.03	49.17	29.31	21.19	25.18	5.73	10.19
16:1	0.83	3.57	6.07	7.49	5.83	8.29	2.89
17:0	0.99	0.70	0.40	0.39	0.26	0.31	0.30
17:1	–[e]	0.31	0.51	0.55	–	–	0.43
18:0	15.46	9.68	3.17	2.89	3.60	1.28	1.75
18:1	4.85	29.50	55.52	44.58	29.34	43.17	27.50
18:2	–	–	0.61	19.04	31.91	37.51	37.41
18:3	–	–	–	–	0.87	1.07	4.37
20:0	0.39	0.20	–	–	–	–	–
20:1	–	–	0.37	0.46	0.26	0.31	0.20
20:3	–	–	–	–	–	–	1.06
20:4	–	–	–	–	–	–	7.34
20:5	–	–	–	–	–	–	1.14
22:0	0.52	–	–	–	–	–	–
22:4	–	–	–	–	–	–	0.67
22:5	–	–	–	–	–	–	0.47
22:6	–	–	–	–	–	–	3.37
24:0	0.41	–	–	–	–	–	–

[a] The sum is not 100%, because trace fatty acids are not included regardless their molecular structure; the sum of position isomers is presented for unsaturated acids, because they are not resolved by capillary GC or DCI-MS.

[b] The symbol, *e.g.*, 012 refers to all TGs with one FA with two double bonds (2), one FA with one double bond (1) and one saturated (0) FA. Other symbols are interpreted in a similar manner.

[c] TLC fractions with equal unsaturation but different fatty acids.

[d] More unsaturated TG fractions, *e.g.* 113, 114, 124, etc.

[e] Trace fatty acids at less than 0.2 mol% are not listed in this table.

much more variable than shown. The occurrence of TGs with an identical summary formula, but formed by different fatty acids, *e.g.* MPS and PPP or PPoO and MOO, was verified on the basis of the balance of FAMEs of a corresponding fraction. However, during separation by silver ion TLC, differences in the molecular structures of the acids involved play a role, and the critical pair can thus be separated, *e.g.* PPoO and PPL or POO and PSL etc.

On the basis of DCI-MS data and retention characteristics, TGs with odd carbon atom numbers could be detected. The existence of these TGs is supported by the presence of the above acids in fractions after silver ion TLC (see Table I; *e.g.* 17:0 and 17:1 acids). Their elution behaviour corresponds to the data published previously [18]. The results of quantitative analysis carried out with both techniques are comparable, as can be seen from Table II. Unfortunately, significant differences were detected in the data for saturated TGs, where it can be assumed that the data obtained by means of DCI-MS are overestimated owing to the ionization energy of the C=C bond being less than that of the C–C bond. This difference was verified by measuring equal masses of the standards (54:0, 57:0 and 60:0).

In order to obtain a reproducible quantitative representation, we calibrated the system using model compounds, both in capillary GC and DCI-MS. It was found that the discrimination of the TGs (by abundance) increased with increasing number of C atoms and C=C bonds. This result is at variance with certain literature data [19] but in agreement with others [20,21]. On the other hand, a previous paper [21] reported good agreement for the majority of peaks, where capillary GC–MS on a non-polar phase and DCI-MS were compared (*e.g.* for 54:4, 21.6 and 20.1% total TGs were detected by DCI-MS and capillary GC–MS, respectively). Our previous measurements performed with mixtures of model TGs with a defined composition demonstrate that, by means of optimization of the DCI-MS conditions, it is possible to remove discrimination of TGs almost completely.

Although DCI-MS alone or with silver ion TLC yields only a summary formula of TGs, DCI-MS is a suitable complementary technique with capillary GC on a polarizable stationary phase (especially for polyunsaturated TGs). Its applicability can be demonstrated by the detection of low concentrations of polyene TGs with carbon number 56, which have not previously been detected owing to significant losses during capillary GC.

TABLE II

ANALYSIS OF TG FROM HUMAN BLOOD BY CAPILLARY GC AND DCI-MS

Carbon number	Quantification	Number of double bond(s) TG fraction											
		0 000		1 001		2 011		2 002	3 111		3 012		
	GC[a]		–		0.09								
44		**MMP**		MMPo									
	MS[b]		–		–								
	GC		0.30	**MMO** +	0.60		0.15						
46		**MPP**		**MPPo**		MPoPo							
	MS		–		–		–						
	GC		0.13										
47		PdPP											
	MS		0.28										
	GC	**MPS** +	0.89	**MPO** +	2.81	**MPoO** +	1.47						–
48		**PPP**		**PPPo**		PPoPo		**MPL**[c]			MPoL		
	MS		1.50		2.82		1.73						0.71
	GC		0.13		0.24								
49		PdPS		PdPoS									
	MS		1.01		0.76								
	GC		0.62		9.03	**MOO** +	9.57	**MSL**[c] +		2.16	**MOL**[c] +		
50		**PPS**		**PPO**		**PPoO**		**PPL**	PoPoO		**PPoP**		
	MS		2.81		9.49		8.65			2.93			
	GC		0.19		0.78		0.16						
51		PdSS		PdSO		PdOO		PdSL[c]					
	MS		0.55		0.85		0.35						
	GC		0.18		3.09		28.48			15.62			
52		**PSS**		**PSO**		**POO**		**PSL**[c]	PoOO		**POL**[c]		
	MS		0.93		3.83		28.94			13.71			
	GC						0.37						
53						HdOO							
	MS						0.65						
	GC		–		0.22		2.31			4.81			2.22
54		**SSS**		**SSO**		**SOO**		SSL[c]	**OOO**		**SOL**[c]		
	MS		–		0.96		2.94			4.90[d]			
	GC												
56													
	MS												

[a] Quantification by capillary GC.

[b] Quantification by DCI-MS.

[c] Not separated in the total sample either by capillary GC or DCI-MS; identified after separation on silver ion TLC in the individual fractions by capillary GC and DCI-MS, therefore the individual peaks were not quantified.

[d] And SLO.

[e] And SLL.

[f] The structure is predicted on basis of DCI-MS only: M = myristic acid; Pd = pentadecanoic acid; P = palmitic acid; Po = palmitoleic acid; Hd = heptadecanoic acid; S = stearic acid; O = oleic acid; L = linoleic acid; Ln = linolenic acid; Ar = arachidonic acid; Dh = docosahexaenoic acid.

$\frac{4}{112}$	$\frac{4}{022}$	$\frac{4}{013}$	$\frac{5}{122}$	$\frac{5}{014}$	$\frac{6}{114}$	$\frac{7+}{124+}$
0.09 PoPoL 0.12	MLL[c]					
3.43 **PoOL** 3.95	**PLL**[c]	POLn[c]				
1.93 **OOL** 2.74[e]	**SLL**[c]	SOLn[c]	**OLL** 0.23 1.26			
				SOAr[f] 0.30	OOAr[f] 0.34	OLAr+ − PODh[f] 0.21

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