Journal of Chromatography, 500 (1990) 427–441
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 094

GAS–LIQUID CHROMATOGRAPHIC PROFILING OF PLASMA LIPIDS USING HIGH-TEMPERATURE-POLARIZABLE CAPILLARY COLUMNS

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(First received August 24th, 1989; revised manuscript received October 16th, 1989)

SUMMARY

We have examined the potential usefulness in plasma lipid profiling of capillary columns coated with a high-temperature-polarizable phenylmethyl silicone liquid phase, previously employed for gas–liquid chromatography (GLC) of natural triacylglycerols. The column yielded an excellent resolution for plasma triacylglycerols, cholesteryl esters and the trimethylsilyl (TMS) and *tert.*-butyldimethylsilyl (*t*-BDMS) ethers of free cholesterol, as well as of the TMS and *t*-BDMS ethers of the diacylglycerols and ceramides released from plasma phospholipids by phospholipase C digestion. The various lipid classes were resolved into molecular species according to chain length and number of double bonds. The different molecular species were recovered in variable proportions as indicated by comparisons of peak area percentages obtained on the polar columns with those recorded on non-polar columns, which have been previously shown to give correct quantitative proportions. The cholesteryl esters are thermally degraded at high temperatures and must be eluted at as low a temperature as possible. Under the selected experimental conditions, the cholesteryl esters were found to partly interdigitate with the triacylglycerol species of carbon numbers 46–48. The TMS and *t*-BDMS ethers of diacylglycerols and ceramides emerged clearly ahead of the leading triacylglycerol of the 42 acyl carbon series. However, the diacylglycerol and ceramide species overlapped and interdigitated extensively with each other. Due to low polarity below 280°C, the polarizable-liquid phase was not suitable for the resolution of the molecular species of monoacylglycerols and free fatty acids, which were separated on the basis of carbon number only. Nevertheless, capillary GLC on the polarizable liquid phases provides a novel and informative profiling of plasma lipids, the application of which to the assay of plasma lipid abnormalities deserves further examination.

INTRODUCTION

Of great potential interest to plasma lipid profiling is the preparation of capil-

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lary columns coated with the polarizable phenylmethylsilicone phase[1]. The liquid phase becomes more polar with increasing temperature and is stable up to 360°C. These columns provide sufficient resolving power for the separation of molecular species of natural triacylglycerols. Geeraert and Sandra[2] have made extensive use of such columns in the analysis of various vegetable oils and animal fats. It has also been shown[3,4] that this column is suitable for direct interfacing with a mass spectrometer. In the present study, we have examined the usefulness of two capillary columns with polarizable liquid phases for the separation of plasma triacylglycerols along with cholesteryl esters, free cholesterol and the diacylglycerols and ceramides released from plasma phospholipids by hydrolysis with phospholipase C and have obtained encouraging results. Preliminary reports have appeared[5,6].

EXPERIMENTAL

Plasma lipids

Plasma samples from normolipemic subjects were available in the laboratory from routine analyses. Small aliquots (0.25 ml) were digested with phospholipase C and the neutral lipid extracts reacted with trimethylsilyl (TMS) or *tert.*-butyldimethylsilyl (*t*-BDMS) reagents as previously described[7] and used for direct analysis with the polar capillary columns. Other aliquots were digested with phospholipase C and subjected to normal phase thin-layer chromatography (TLC)[8] for the resolution of monoacylglycerols, ceramides, diacylglycerols, free cholesterol, free fatty acids, triacylglycerols and cholesteryl esters. The partial acylglycerols, ceramides, free cholesterol and the free fatty acids were silylated[7] before analysis by gas–liquid chromatography (GLC). Still other aliquots of plasma were subjected to total lipid extraction followed by resolution of neutral and polar lipids by TLC[9].

TLC

Neutral and polar lipids were resolved using heptane–isopropyl ether–acetic acid (60:4:4) as the developing solvent[8], which retained the phospholipids at origin. The neutral lipids were recovered by extracting the gel scrapings with chloroform–methanol (2:1), while the polar lipids were recovered by extraction with chloroform–methanol–water–acetic acid (150:117:30:3)[9]. The ceramide and diacylglycerol moieties released from the phospholipids by phospholipase C were resolved by TLC on borate-treated silica gel using chloroform–acetone (97:3) as developing solvent and appropriate standards as area markers.

GLC

The analyses were done on a Hewlett-Packard Model 5880 gas chromatograph equipped with a hydrogen flame ionization detector and an on-column injector for capillary columns (Hewlett-Packard, Palo Alto, CA, U.S.A.). Analyses by carbon number were performed using a flexible quartz column (8 m × 0.32 mm I.D.) coated with a permanently bonded SE-54 liquid phase (Hewlett-Packard) as previously described[10]. Analysis by carbon and double bond number were performed using a flexible quartz column (25 m × 0.25 mm I.D.) coated with methyl 50% phenylsilicone film (RSL-300) custom made by one of us (P.S.) and on a commercially available triglyceride column (25 m × 0.25 mm I.D.) coated with methyl 65% phenyl-

silicone film (OV-22 equivalent, Quadrex, New Haven, CT, U.S.A.). Both columns were conditioned under hydrogen carrier flow, the RSL-300 for 48 h as previously described[4] and the OV-22 column for several h. Samples were injected using either a split injector at 330°C or manually on column at 40°C. Various temperature programs were used but typically, after 1 min the column was heated ballistically either to 220°C or 290°C and, when the microprocessor controller indicated that the column was ready (equilibration time, 0.5 min), the integration plot was initiated. After an adjustment of 0.5 min, the temperature was programmed at various rates to 330°C and then at 2°C/min to 350°C. Single column compensation was used to correct for column bleed. The carrier gas was hydrogen at 0.5 to 1 bar. The methyl esters of fatty acids, the TMS ethers of diacylglycerols and cholesteryl esters were analyzed on a 15 m × 0.32 mm I.D. flexible quartz column (RTx 2330, Restex Corp., Port Matilda, PA, U.S.A.) as described[6,11].

GLC–mass spectrometry (MS)

This was performed with a Hewlett-Packard Model 5985B quadrupole instrument interfaced with the RSL-300 polarizable capillary column as previously described[4]. Electron impact (EI) spectra were recorded at 70 eV and a hydrogen carrier gas head pressure of 0.34 bar. The data were analyzed by means of a Hewlett-Packard data system (Model HP 1000) and a graphics terminal (Model HP 2648A) as previously described[12].

RESULTS

Resolution of triacylglycerols and cholesteryl esters

Fig. 1 gives the triacylglycerol profile of a normal plasma as obtained using the

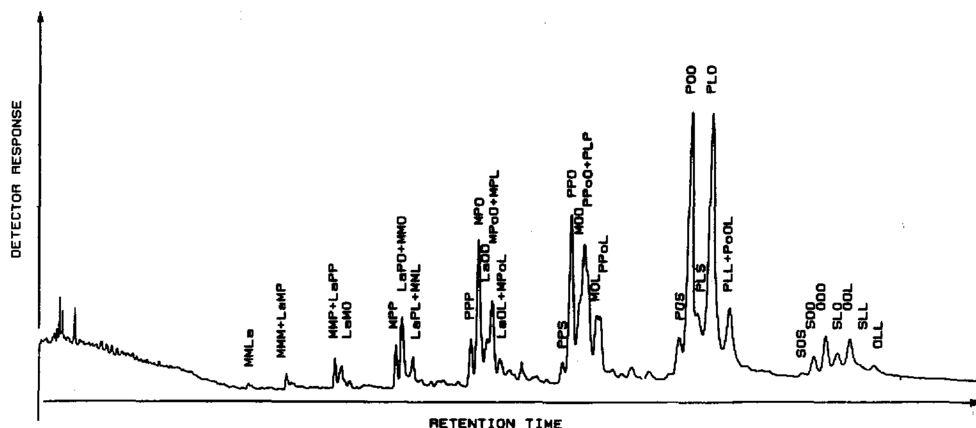

Fig. 1. Polar capillary GLC of plasma triacylglycerols from a normolipemic subject. Peak identification as given in figure: La = lauric; L = linoleic; M = myristic; O = oleic; P = palmitic, Po = palmitoleic; and S = stearic acids. GLC conditions: column, fused-silica capillary (25 m × 0.25 mm I.D.) coated with 50% phenylmethylsilicone (RSL-300); temperature program: 40–290°C, ballistic; 290°C, isothermal for 0.5 min; 290–330°C, 10°C/min; 330–360°C, 1°C/min. Trioleoylglycerol retained 21.23 min. Carrier gas, hydrogen at 1 bar head pressure. Manual on-column injection with fused-silica needle at 40°C. Instrumentation and other GLC conditions as given in text. Sample: 0.2 μl of a 0.1% solution in hexane.

RSL-300 polarizable capillary column. The triacylglycerols, which are eluted over a wide temperature range, have been identified by reference to standard monoacid triacylglycerols, and from the knowledge of the fatty acid composition of the sample[13], as well as by reference to Geeraert and Sandra[2]. It is seen that the plasma triacylglycerols are resolved on the basis of molecular weight and degree of unsaturation very much like the triacylglycerols of adipose tissue and vegetable oils and fats[2]. However, many major species remain unresolved and many more minor ones remain unidentified. Within each carbon number the saturated species are eluted first followed by mono-, di-, tri- and more unsaturated species in order of increasing retention time. In all instances, the most unsaturated species of the preceding carbon number are completely eluted before the saturated species of the next highest even carbon number emerges. Table I compares the relative recoveries of the triacylglycerols by carbon number from the polar RSL-300 column and from a non-polar SE-54 column to the carbon numbers calculated for the triacylglycerols on the basis of a 1-random 2-random 3-random association of the fatty acids of known positional distribution[14]. There is a reasonably close agreement in the recoveries of the triacylglycerols up to and including C_{52} species. The C_{54} triacylglycerol species, however, are only partially recovered (about 50%). Table II compares the composition of the molecular species resolved by the RSL-300 column and the composition calculated on the basis of the knowledge of the 1-random 2-random 3-random structure of the triacylglycerol of the sample. There appears to be a reasonably close agreement between the determined and calculated values, except for carbon number 54. In addition to the general loss of these higher molecular weight triacylglycerols, there has been a

TABLE I

RECOVERY OF TRIACYLGLYCEROLS BY CARBON NUMBER FROM POLAR AND NON-POLAR CAPILLARY COLUMNS DURING HIGH-TEMPERATURE GLC

Representative analyses.

Carbon number	*Area %*		
	GLC analyses		*Calculated*
	RSL-300	*SE-54*	
40			
42	0.4	0.4	0.1
43			
44	1.6	1.3	0.6
45		0.4	
46	4.7	4.1	2.9
47		0.6	0.3
48	13.5	11.1	9.5
49		1.5	1.0
50	24.8	22.9	24.2
51		1.6	1.4
52	43.2	43.0	43.7
53			0.5
54	7.0	13.0	12.9
56		1.1	1.3

TABLE II

COMPOSITION OF MOLECULAR SPECIES OF PLASMA TRIACYLGLYCEROLS AS ESTIMATED BY POLAR CAPILLARY GLC AND CALCULATION

Representative analyses.

Carbon number	*Molecular species*	*GLC*	*Calculated*
42:0	MMM + LaMP	0.4	0.1
42:1			0.03
44:0	MMP + LaPP	0.7	0.3
44:1	LaMO	0.9	0.3
44:2			0.1
46:0	MPP	1.1	0.7
46:1	LaPO + MMO	2.4	1.4
46:2	LaPL + MML	1.4	0.7
46:3		0.4	0.1
48:0	PPP	1.4	1.2
48:1	MPO	5.5	4.2
48:2	LaOO + MPoO + MPL	4.9	3.0
48:3	LaOL + MPoL	1.2	0.9
48:4			0.1
50:0	PPS	0.8	0.5
50:1	PPO	6.8	8.6
50:2	MOO + PPoO + PLP	11.8	9.9
50:3	MOL + PPoL	4.8	4.2
50:4			0.9
50:5			0.1
52:1	POS	2.2	2.5
52:2	POO + PLS	17.3	17.7
52:3	PLO	17.9	16.7
52:4	PLL + PoOL	5.7	5.7
52:5			1.0
54:1	SOS	0.3	0.2
54:2	SOO + SLS	0.9	2.2
54:3	SLO + OOO	3.4	3.9
54:4	OOL + SLL	2.3	3.4
54:5	OLL	0.45	2.46
54:6			0.9

progressive loss of the molecular species with increasing degree of unsaturation. As a result the composition of these species has been distorted in favour of the less unsaturated components.

Fig. 2 shows the elution pattern of the triacylglycerols and cholesteryl esters of another sample of plasma. These triacylglycerols possess a triacylglycerol pattern that differs significantly from that in Fig. 1, but all the species can be readily recognized from the retention times and by reference to the pattern in Fig. 1. Some of the lower molecular weight triacylglycerols are largely obscured by the cholesteryl ester peaks with which they overlap or interdigitate. The major peaks can be clearly identified as cholesteryl myristate, palmitate, palmitoleate, stearate, oleate and linoleate. There is

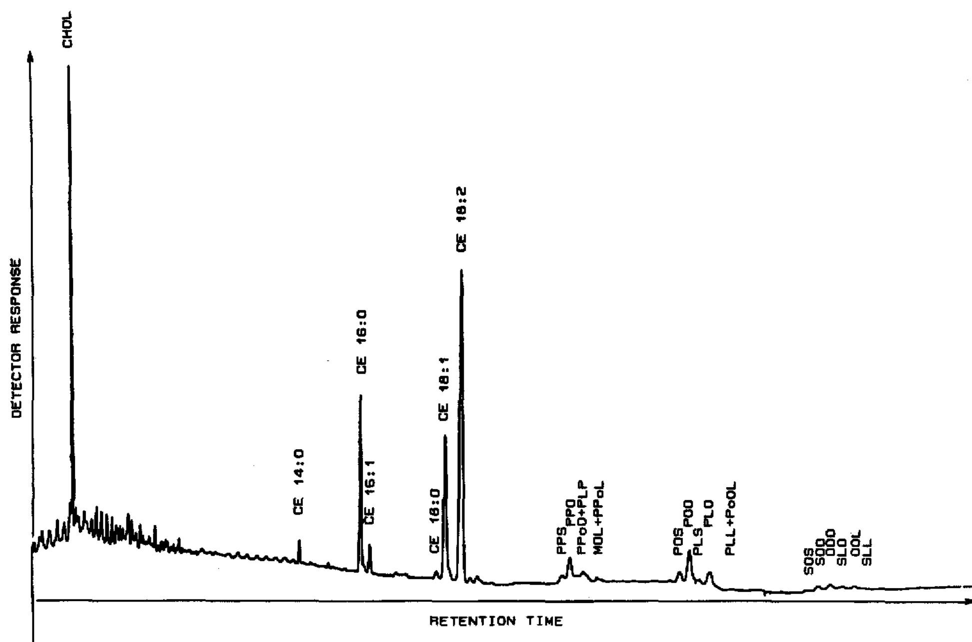

Fig. 2. Polar capillary GLC of plasma neutral lipids from a normolipemic subject. Chol = free cholesterol; CE 14:0–CE 18:2 = cholesteryl esters; other peaks identified as in Fig. 1. Column and GLC conditions as given in Fig. 1. Sample 0.2 μl of a 0.05% solution in hexane.

no discernible peak for arachidonate, which was a minor component in this plasma. The 18:2 fatty acid ester of cholesterol overlaps with tripalmitoylglycerol, while the 18:0 and 18:1 esters are clearly resolved from the triacylglycerols and emerge well before the 46:0 species, which is the earliest eluted triacylglycerol of the C_{46} series. Likewise, the 16:0 and 16:1 esters emerge clearly between 44:1 and 44:0 triacylglycerol series. The C_{15} and C_{17} cholesteryl esters, however, overlap completely with the odd carbon number triacylglycerols C_{45}, C_{43} and C_{41}, respectively. Table III compares the relative recoveries of the major cholesteryl esters from two polarizable capillary columns (RSL-300 and OV-22) and another polar capillary column (RTx 2330)[6] to the values determined from the fatty acid analyses. There has been an extensive loss of the minor 20:4 and to a lesser extent of the 18:2 ester fraction. There appears to be a decomposition of all cholesteryl esters due to the high temperature of elution. As illustrated in Fig. 3 by means of a higher sample load, there is a gradual build-up of the baseline due to some ill-defined material, which drops suddenly when the last major cholesteryl ester peak is eluted. In relation to the triacylglycerols, the cholesteryl esters appear to be recovered at 50% of the anticipated proportion. The other 50% is present in the hump containing the degraded cholesteryl esters. This type of degradation was experienced also with the OV-22 column and with both cold on-column and split injector. This indicates that runs made on the RSL-300 and the OV-22 columns should be made under conditions that minimize the elution temperatures and times to avoid cholesteryl ester degradation.

TABLE III

RELATIVE RECOVERIES OF CHOLESTERYL ESTERS FROM POLAR CAPILLARY GLC AND CALCULATION FROM FATTY ACID COMPOSITION

Representative analyses. nd = Not detected.

Cholesteryl esters	*Intact esters*			*Fatty acids*
	RSL-300	*OV-22*	*RTx 2330*	
14:0	1.8	1.9	0.9	1.4
15:0	0.4	0.5	0.3	0.4
16:0	18.6	20.0	12.8	13.5
16:1	3.1	4.4	3.8	4.5
17:0	nd	0.3	nd	0.2
18:0	1.8	2.2	1.6	1.7
18:1	23.0	24.9	26.8	24.8
18:2	51.3	45.9	48.9	48.2
18:3	nd	nd	0.4	0.4
20:4	nd	nd	4.4	4.8
22:6	nd	nd	nd	0.1

Resolution of TMS ethers of diacylglycerols and ceramides

Both, RSL-300 and OV-22 polarizable capillary columns are also capable of resolving the molecular species of the diacylglycerol moieties of plasma phosphatidylcholine (PC) and the ceramide moieties of the sphingomyelins as either the TMS

Fig. 3. Polar capillary GLC of plasma neutral lipids from a normolipemic subject. Peak identification, column and GLC conditions as in Fig. 1. Sample: 0.2 μl of a 0.3% solution in hexane.

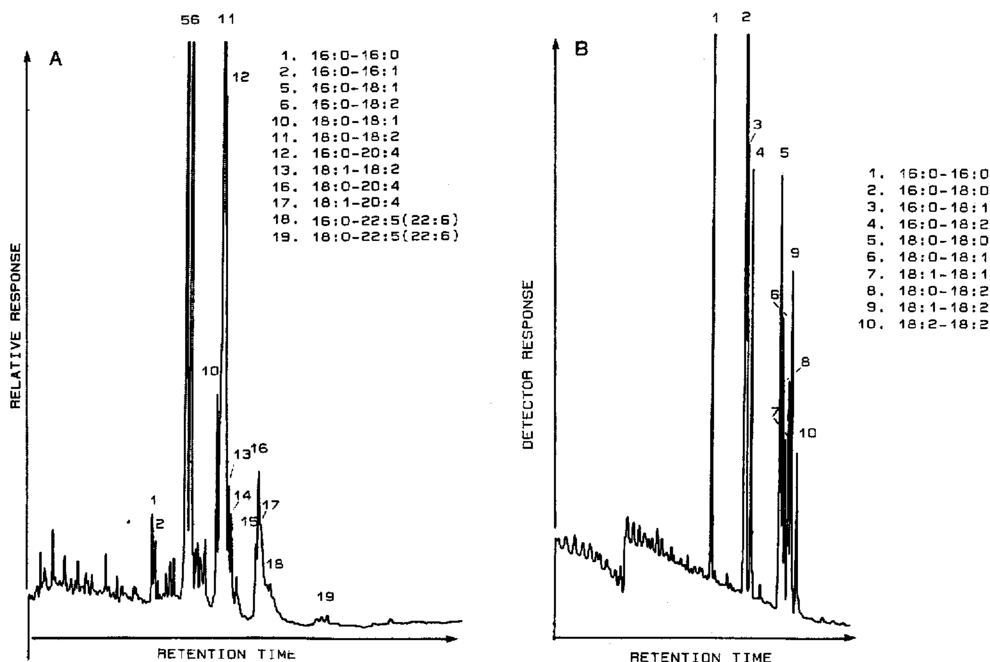

Fig. 4. Polar capillary GLC of the diacylglycerol moieties of plasma phosphatidylcholine (A) and of a synthetic mixture of diacylglycerols (B) as the *t*-BDMS ethers. Tentative peak identification as given in figure. Temperature program: (A) as given in Fig. 1, except 300–340°C at 10°C/min, then 1°C/min; (B) as in Fig. 1. Sample: 0.2 μl of a 0.1% solution in hexane.

or the *t*-BDMS ethers. Fig. 4 shows the resolution of the *t*-BDMS ethers of the diacylglycerol moieties of PC of a normolipemic plasma along with a mixture of synthetic diacylglycerols on the RSL-300 column under the conditions of triacylglycerol separation. At the selected column temperature (320–340°C), the column possesses sufficient polarity to effect separations based on both carbon and double bond number. There is an effective resolution of the oligoenoic species in both samples, but the polyunsaturated species appear to make the elution pattern complicated. There may have been some loss and degradation. Experience with other polar columns has shown that 16:0–20:4 may not be well resolved from 18:0–18:2 if polarity is not high enough and 16:0–20:3 can be eluted after 16:0–20:4. As the column polarity increases, 16:0–20:4 is well separated from 18:0–18:2, and 16:0–20:3 and 16:0–20:4 merge. The elution order of mixtures containing polyunsaturated diacylglycerols is not clearly established for polarizable columns at present. However, all the diacylglycerol species are eluted ahead of the lowest-molecular-weight triacylglycerol species found in normal plasma from subjects on normal diets. Table IV compares the recoveries of the diacylglycerol moieties of plasma PC from the RSL-300 column and from the RTx 2330 capillary column[15]. There is a good agreement between the proportional estimates of the oligoenoic species and fair agreement for the sums of the polyenoic species.

Fig. 5 shows the elution profile of the ceramide moieties of plasma sphingomyelin as the *t*-BDMS ethers on the RSL-300 column using the temperature program and

TABLE IV

RELATIVE RECOVERIES OF DIACYLGLYCEROL MOIETIES OF PLASMA PHOSPHATIDYLCHOLINE BY POLAR CAPILLARY GLC

Representative analyses.

Molecular species	*Area%*			
	GLC analyses			
	OV-22		*RTx 2330*	
16:0–16:0	1.1		0.9	
16:0–16:1	0.6		0.6	
16:0–18:1	16.1		14.6	
16:0–18:2	27.8		25.0	
18:0–18:1	3.6		4.3	
18:0–18:2			20.4	
16:0–20:4			6.6	
16:0–20:3		35.8	3.1	35.4
18:1–18:2			3.6	
16:0–20:5			0.4	
18:2–18:2			1.3	
18:0–20:4			5.7	
18:0–20:3			2.6	
18:1–20:4 + 18:1–20:3			1.5	
18:0–20:5		9.8	0.5	12.5
16:0–22:6			1.1	
18:0–20:5			0.6	
18:2–20:4			0.5	
18:0–22:6		nd	0.4	0.7
18:0–22:5			0.3	

flow-rate optimized for triacylglycerol separation. There is an excellent separation of all molecular species. Table V compares the recovery of the ceramide species from the RSL-300 and a non-polar capillary column[16]. There has been an effective recovery of all species on the basis of carbon number. The values for actual species are consistent with analyses of similar samples by GLC–MS[16]. Although the *t*-BDMS ethers of the ceramides interdigitate extensively with the lower-molecular-weight triacylglycerols and cholesteryl esters, the TMS ethers are eluted prior to cholesteryl myristate and the triacylglycerols.

However, there is extensive overlapping and interdigitation of the ceramides and diacylglycerols and it occurs with both TMS and *t*-BDMS ethers. However, the relative order of elution depends on the derivative. Thus, as the *t*-BDMS ether, the ceramide d18:1–16:0 (a major component) is eluted between the diacylglycerols 16:0–16:0 and 16:0–18:1, and the C_{42} ceramides overlap with the 18:0–22:5(22:6) diacylglycerols. When TMS ethers are used, d18:1–16:0 is eluted ahead of 16:0–16:0 and the C_{42} ceramides overlap with 16:0–22:5(22:6).

The monoacylglycerols and free fatty acids also present in the plasma neutral lipid fraction are resolved without overlapping, but only on the basis of carbon number or molecular weight, because they are eluted below a column temperature of

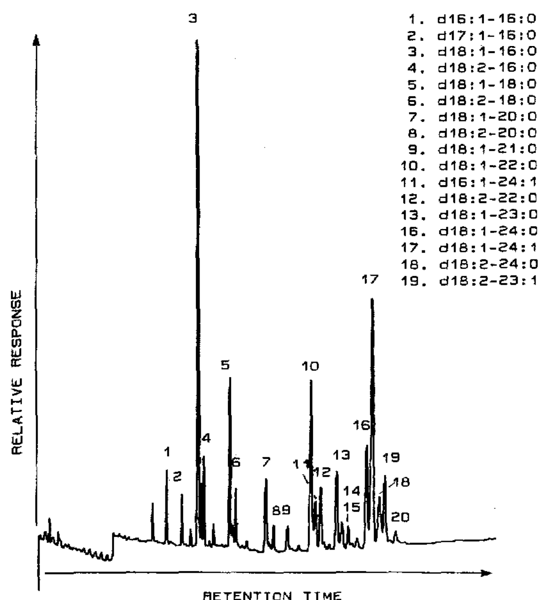

Fig. 5. Polar capillary GLC of the ceramide moieties of plasma sphingomyelin as the *t*-BDMS ethers. Peak identification as given in figure. Column and GLC conditions as in Fig. 1. Sample: 0.1 μl of a 0.1% solution in hexane.

280°C, where this liquid phase has insufficient polarity for differential retention of molecular species differing in unsaturation.

Fig. 6 shows the plasma total lipid profile obtained on yet another plasma sample using the OV-22 polarizable capillary column utilizing the TMS ethers of the diacylglycerols and ceramides. A highly complex peak pattern is obtained, which, however, can be readily interpreted in terms of the major components as indicated in the chromatogram. The sample was injected on-column at 40°C and reveals the presence of free cholesterol and tridecanoylglycerol as internal standard in addition to the other components just discussed. The resolution of the various molecular species is closely similar to that described for the RSL-300 column, except that in this instance all the plasma lipid classes are injected as a mixture. Clearly, similar interdigitation and peak overlapping is present. Table VI compares the recoveries of the major components from the OV-22 polarizable capillary column and from a short non-polar capillary column run in parallel. While the lower molecular weight components are recovered in a reasonably close approximation to those obtained on the short non-polar capillary column, the higher molecular weight polyunsaturated triacylglycerols and especially the cholesteryl esters have experienced significant losses, which would have to be corrected or compensated for in a quantitative analysis. In relation to the internal standard the overall recovery of the cholesteryl esters was about 60%, ranging from 73% for C_{43} (palmitate and palmitoleate) to 56% for C_{45} (oleate and linoleate) while C_{47} (arachidonate) was not visible. The recovery of the triacylglycerols ranged from 50% for the C_{54} species to about 100% for the C_{50} and lower-molecular-weight components. The chromatographic patterns of the peaks were easily reproduced in the limited number of trials made with the OV-22 column. Thus, the

TABLE V

RELATIVE RECOVERIES OF MOLECULAR SPECIES OF THE CERAMIDE MOIETIES OF PLASMA SPHINGOMYELINS FROM RSL-300 AND SE-54 CAPILLARY COLUMNS

Representative analysis.

Molecular species	*Area %*		
	Method of analysis		
	RSL-300	*RSL-300*	*SE-54*
32		1.9	1.7
d18:1–16:0	1.9		
33		1.8	1.2
d17:1–16:0	1.2		
34		28.1	28.2
d16:1–18.0	0.4		
d18:1–16:0	25.0		
d18:2–16:0	2.7		
35		0.8	0.9
d18:1–17:0	0.8		
36		8.5	8.9
d16:1–20:0 } d18:1–18:0 }	6.4		
d18:2–18:0	2.1		
37		0.7	0.5
	0.1		
	0.3		
	0.1		
38		5.2	5.3
d16:1–22:0	3.8		
d18:1–20:0	1.3		
d18:2–20:0	0.1		
39		1.4	1.4
d17:1–22:0	1.0		
	0.1		
	0.2		
40		14.5	14.5
d18:1–22:0	8.4		
d16:1–24:1	2.3		
d18:2–22:0	3.0		
41		6.4	6.3
d17:1–24:0	3.2		
d17:1–24:1	1.2		
d18:1–23:0	0.6		
d18:2–23:0	1.1		
42		29.4	31.2
d18:1–24:0	6.0		
d18:1–24:1	14.5		
d18:2–24:0	4.2		
d18:2–24:1	4.8		
d18:2–24:2	1:1		

methyl 50% phenylsilicone RSL-300 and the methyl 65% phenylsilicone OV-22 columns appear to possess good potential for detailed determination of plasma lipid profiles. Since the resolution, recovery and indeed degree of degradation of the components varies with each column and working conditions, an appropriate quantitative response would have to be worked out for each column and for each set of working conditions.

DISCUSSION

Geeraert and Sandra[2] have discussed the application of the methyl 50% phenylsilicone columns for the separation of a variety of natural triacylglycerol fats and oils. They have noted that these columns give essentially complete resolution and recoveries of molecular species on the basis of carbon and double bond number, although the polyunsaturated fish oil triacylglycerols may be too complex and may also suffer degradation. We have shown that this column is suitable also for the separation of

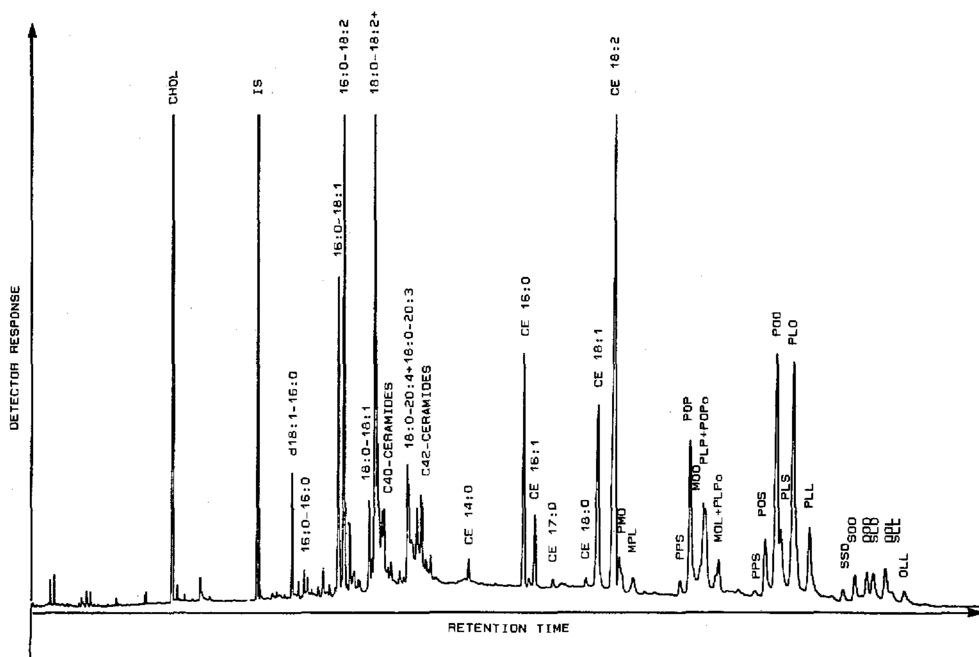

Fig. 6. Polar capillary GLC profile of plasma total lipids from a hyperlipemic subject following dephosphorylation and trimethylsilylation. IS = internal standard (tridecanoylglycerol); 16:0–16:0 etc. = diacylglycerols; d18:1–16:0 etc. = ceramides; other peaks identified as in Figs. 1 and 2. Column, fused-silica capillary (25 m × 0.25 mm I.D.) coated with methyl 65% phenylsilicone (OV-22); temperature program, 40–220°C, ballistic; 220–320, 10°C/min, then to 360°C at 2°C/min. Carrier gas, hydrogen at 1 bar head pressure. Trioleoylglycerol retained 24.6 min. Instrumentation and other GLC conditions as given in text. Sample: 0.2 μl of a 0.1% solution in hexane.

TABLE VI

RELATIVE RECOVERIES OF PLASMA LIPID CLASSES AND MOLECULAR SPECIES FROM POLAR AND NON-POLAR CAPILLARY COLUMNS

Representative analysis. TD = tridecanoylglycerol.

Lipid classes and molecular species	*Area/TD GLC analyses*		*Recovery (%)*
	OV-22	*SE-54*	
Free cholesterol	229.7	232.6	98.7
TD	100	100	100
d18:1–16:0	15.2	31.0	48.9
C_{34} Diacylglycerols	173.8	184.4	94.2
C_{36} Diacylglycerols	180.5	223.9	80.6
C_{16} Cholesteryl esters	72.3	98.9	73.1
C_{18} Cholesteryl esters	239.7	423.4	56.6
C_{50} Triacylglycerols	150.1	150.1	100
C_{52} Triacylglycerols	335.2	377.9	88.7
C_{54} Triacylglycerols	69.7	135.6	51.4

plasma triacylglycerols[5]. Mares[17] has reported similar results with other samples of plasma triacylglycerols, as well as has noted that the C_{16} cholesteryl esters are eluted between the $C_{46:1}$ and C_{48} triacylglycerols, while the C_{18} esters are recovered between $C_{48:0}$ and $C_{48:2}$ triacylglycerols. Mares[17] noted considerable losses of cholesteryl arachidonate and other polyenoic cholesteryl esters and suggested that a capillary length of 25 m may not be suitable for the analysis of polyenoic cholesteryl esters. The present study confirms the observations of Mares[17] in regards to the adverse effects of high temperature on the recovery of the cholesteryl esters, but also demonstrates that recoveries can be improved by lowering the working temperatures and by increasing flow-rates, which allows shortening of retention time. We have shown elsewhere[6] that prolonged elution times can lead to possible thermal modification of cholesteryl arachidonate even at 260°C when run on polar capillary columns.

The problem of peak overlapping with triacylglycerols remains and cannot be solved, unless a mass spectrometer is employed as a chromatographic detector[18]. In non-polar capillary GLC–MS, the steryl esters yield readily detectable ions for the steroid skeleton of the cholesteryl ester molecule. This permits the identification and quantitation of cholesteryl esters overlapping with triacylglycerols. The triacylglycerols are readily recognized and quantitated on the basis of the fragments representing the diacylglycerol ions and the molecular weight of the molecule. We have previously demonstrated[4] the suitability of the polarizable capillary column for the detection of cholesterol and triacylglycerol fragments in butteroil distillates.

There have been no previous reports on the utilization of the polarizable capillary GLC columns for the resolution of molecular species of natural diacylglycerols or ceramides. Since these columns develop significant polarity only above 280°C, it is necessary to operate them above this temperature to obtain effective resolution on the basis of double bond number. In the present study effective separations of synthetic and natural diacylglycerols were obtained in the range 320–340°C using the temperature program optimized for resolution of triacylglycerols. At this temperature, however, there is some loss or alteration of the polyunsaturated species. This was indicated by both decreased overall recovery and poor resolution of the polyunsaturates. On the RTx 2330 capillary GLC column, we have obtained quantitative resolution of all molecular species of diacylglycerols at 250–260°C over a period of about 30 min, when run as the TMS[15] or *t*-BDMS[19] derivatives. Clearly, the time and temperature of elution must be kept to a minimum compatable with sufficient polarity of the column for accurate work with both triacylglycerols and diacylglycerols. There is no serious overlapping between plasma triacylglycerols and the diacylglycerol moieties of plasma phosphatidylcholines, when run as TMS or *t*-BDMS ethers.

The polarizable capillary GLC columns appear to be especially well suited for the resolution of the molecular species of ceramides both as TMS and *t*-BDMS derivatives. There is no evidence for decomposition or loss of the higher-molecular-weight or more unsaturated species. In the past they have been determined by combining non-polar capillary GLC with MS[15,16,20]. The TMS ethers of the ceramides offer certain advantages over the *t*-BDMS ethers, when run in mixture with other plasma lipids. Thus, they overlap less extensively with the minor low-molecular-weight triacylglycerols than the *t*-BDMS ethers, which are eluted about four methylene units later. However, both TMS and *t*-BDMS ethers of ceramides overlap exten-

sively with the corresponding derivatives of diacylglycerols. The overlap of the major species is less extensive when both ceramides and diacylglycerols are run as the TMS ethers. It must be noted, however, that the exact retention times and peak overlaps differ somewhat between the two columns and between different rates of temperature programming and head pressures. Nevertheless, even under the present chromatography conditions, which have been optimized for the separation of triacylglycerols, useful diacylglycerol and ceramide profiles are obtained. Free cholesterol is recovered as a single sharp peak when run either as the TMS or *t*-BDMS derivative without any evidence of decomposition or loss. Free fatty acids and monoacylglycerol TMS and *t*-BDMS derivatives are recovered in the temperature range (below 280°C), where these capillary columns possess little polarity[2,4] and therefore are resolved on the basis of molecular weight only.

The recovery of the triacylglycerols and the cholesteryl esters varied with the mode of injection and the nature of the temperature program and flow-rate, but without apparent significant difference between the RSL-300 and the OV-22 columns, both of which were of about the same length and film thickness. There were small differences in the resolution of the molecular species between the two columns due to a polarity difference. The slightly more polar methyl 65% phenylsilicone (OV-22) column gave slightly larger separation factors between species than the RSL-300 column.

Both columns yield wide intervals between the elution times of several major components, where no plasma lipids are eluted. These spaces can be utilized to accommodate appropriate internal standards to correct for losses of species of higher molecular weight or higher unsaturation. In the present study, only tridecanoylglycerol was included as an internal standard to determine the relative recoveries of the different lipid classes from the OV-22 column in relation to the SE-54 capillary column of short length.

The polarizable capillary columns should be equally well utilized for the analysis of plasma neutral lipids following a preliminary removal of the phospholipids[2,4]. Alternatively, the plasma phosphatidylcholines can be subjected to a double digestion to yield monoacylglycerols and free fatty acids, which would permit resolution of ceramides without overlap with diacylglycerols[21]. These approaches, however, make it impossible to obtain accurate estimates of the phosphatidylcholine–free cholesterol ratio, which has proved of clinical interest[22]. Finally, the cholesteryl esters could be destroyed by a preliminary digestion with cholesteryl esterase[23], which would allow only total cholesterol estimation, thus seriously compromising the plasma lipid profile.

In conclusion, the polarizable capillary GLC columns clearly provide new insights into the composition of plasma lipids. A simple summation gives an estimate of over 100 resolved peaks, which compares to less than 50 peaks on the non-polar capillary column. The improvement in the resolution is seen in all glyceryl esters and ceramides. Even the cholesteryl esters may be accommodated in the elution pattern when their degradation is minimized. These observations are most encouraging and should stimulate further development of polarizable capillary columns and additional attempts at practical applications in clinical and biochemical research.

ACKNOWLEDGEMENTS

These studies were supported by grants from the Heart and Stroke Foundation of Ontario (Toronto, Canada) and the Medical Research Council of Canada (Ottawa, Canada).

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