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DENSITOMETRIC IDENTIFICATION OF TRIGLYCERIDES SEPARATED BY REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A method to identify triglycerides, separated by reversed-phase thin-layer chromatography on silanized Kieselguhr G layers using densitometric data only, is described. Different triglyceride mixtures, containing components with partition numbers in the range 44–56, have been scanned. A linear relationship between the partition numbers of the zones and the maximum-to-maximum distances of the corresponding peaks, relative to the peak of the zones with partition number 48, has been found. The validity of this equation has been confirmed experimentally for triglycerides with partition numbers in the range 30–46. The method allows the identification of the triglyceride components of a mixture by their partition numbers, provided only one partition number is known or can be determined independently. An example concerning sunflower oil triglycerides is presented.

INTRODUCTION

The identification of triglyceride (TG) groups, separated by reversed-phase thin-layer chromatography (RP-TLC), is a tedious and time-consuming procedure. Usually it is carried out by isolation of the components, transmethylation and analysis of the fatty acids (FAs) by gas–liquid chromatography (GLC). On the other hand, it is often sufficient to obtain only general information on the total TG group composition of the sample, determining the partition numbers (PNs) of the components. In these cases, simultaneous development with suitable reference TG species would be very useful. However, such references are either not available or very expensive.

The combination of RP-TLC with densitometry, developed for the quantification of the separated TG groups[1], offers a possibility for identification of the TG components. The modern scanning densitometers yield not only the usual data for the area/concentration relationship, but give additional information on the migration of the zones along the scan axis Y, recording the positions of peak maxima along this axis. After scanning a great number of resolved TG classes of various natural oils[1–3], we noticed that the distance between the peak maxima of two adjacent zones is very reproducible. This led us to the idea to utilize the scanning data on the migration of the zones for identification purposes, providing that a correlation exists between the maximum-to-maximum distances of the peaks and the corresponding PNs.

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The present paper offers a method for the identification of TG groups in a TG mixture, separated by RP-TLC on silanized Kieselguhr G, based only on densitometric data. This method is applied to the determination of the TG composition of a sunflower oil sample.

EXPERIMENTAL

All solvents were reagent grade and were distilled before use. Chloroform was treated to remove the stabilized alcohol, when used as a component of a developing solvent. Dimethyldichlorosilane was obtained from Fluka. Kieselguhr G (Merck, Darmstadt) was purified before use by washing with chloroform–methanol (1:1, v/v)[3].

Densitometric data for the following TG classes were employed in the present work: S_2M, SM_2, S_2D, SMD, D_2S (where S = saturated, M = monoenoic, D = dienoic fatty acids). These classes were isolated from the purified TGs of commercial sunflower, olive and peanut oils, and lard. Purified TGs of coconut oil were also used. The TG group composition of each class was previously determined by RP-TLC[1–3]. It was checked by GLC, and compared with literature data. The sunflower oil analyzed in this work was also of commercial origin.

Purified TGs from the oils were obtained by preparative TLC on 20 cm × 20 cm plates with a 1-mm thick silica gel layer and a mobile phase of hexane–aetone (100:12, v/v). Pure TG classes were isolated by preparative TLC on 20 cm × 20 cm plates with a 1-mm thick layer of silica gel G containing 5% silver nitrate and a mobile phase of chloroform–diisopropyl ether–acetone, as described elsewhere[3].

Each TG class was separated into TG groups, differing in PNs, by quantitative RP-TLC on a silanized Kieselguhr G layer with a mobile phase of acetone–acetonitrile–water. The specific chromatographic conditions needed for the resolution of each TG class are described in the corresponding papers[1–3].

The analysis of the sunflower oil sample was carried out by a combination of quantitative silver nitrate TLC[4], preparative silver nitrate TLC[2] and quantitative RP-TLC[1].

The GLC analysis of the FAs was performed, after methylation according to Christie[5], on a JEOL-K20 gas chromatograph with 3 m × 3 mm I.D. column containing Chromosorb WAW (60–80 mesh) coated with 8.5% OV-275 and at a column temperature of 190°C.

The densitometric measurements were performed on a Shimadzu CS-930 densitometer by zigzag scanning in a transmission mode at 450 nm and a slit of 1.2 mm × 1.2 mm.

RESULTS AND DISCUSSION

Each of the TG classes investigated contains TG groups with equal numbers of double bonds, determined by the presence of two unsaturated FAs, oleic ($C_{18:1}$) and linoleic ($C_{18:2}$). Consequently the PNs of the TG groups in the respective class depend only on the chain length of the saturated FA. For the selected classes these are: myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), arachidic ($C_{20:0}$), bechenic ($C_{22:0}$) and lignoceric ($C_{24:0}$) acids. Each class contains a TG group with PN = 48. Using the densitometric data for the migration of a TG zone, Y(mm), we determined ΔY(mm),

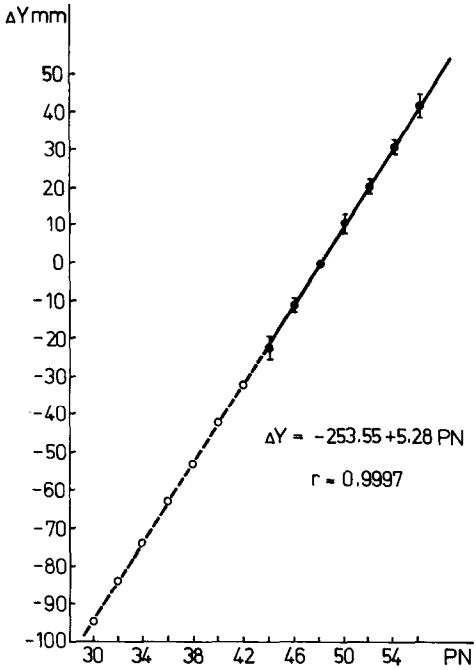

Fig. 1. Plot of the maxima-to-maxima distances of the densitometric peaks, relative to the peak of a zone with partition number 48, *vs.* their partition numbers.

i.e., the distance between the peak maximum of this zone and the peak maximum of the zone with PN = 48. TG groups in the 44–56 range were included. The statistical evaluation of a large number of data revealed that the ΔY values do not depend on the origin or the unsaturation of the TG classes. Thus the mean ΔY value ($\overline{\Delta Y}$) for a TG with a given PN with respect to the TG with PN = 48 can be determined. A linear

TABLE I

THE ΔY *VS.* PN RELATIONSHIP FOR COCONUT OIL TRIGLYCERIDES

PN	ΔY *(mm)*	
	Calc.	*Found**
30	−95.2	−97.4 ± 3.3
32	−84.6	−86.6 ± 1.6
34	−74.0	−75.9 ± 2.5
36	−63.5	−65.1 ± 3.7
38	−52.9	−52.6 ± 3.2
40	−42.4	−41.8 ± 3.5
42	−31.8	−33.2 ± 2.6
44	−21.2	−21.2
46	−10.7	−11.9 ± 1.4

* Mean ± S.D., $N = 7$.

relationship was obtained when ΔY was plotted against the corresponding PN, Fig. 1. The coefficients of the linear equation were determined by the least squares method.

A question arises as to the validity of the established relationship of ΔY *vs.* PN in the case of TG groups having PNs lower than 44. We calculated the theoretical values of ΔY, corresponding to the TGs with PNs from 30 to 42 (the dotted part of the line in Fig. 1). The pure TG from coconut oil, containing TG groups in the range 30–46, was used as a test mixture. From the corresponding densitograms we determined the distance between the peak maxima with respect to the peak maximum of the zone with PN 44. The data are presented in Table I. The statistical treatment with the t-criterion at a significance level of 0.05 indicates that there is a good agreement between the calculated and the experimental data for ΔY.

The experimental relationship between ΔY and PN was applied to identify TG components in a sunflower oil sample. The RP-TLC of the TG classes of this sample

TABLE II

IDENTIFICATION OF TRIGLYCERIDE GROUPS IN SUNFLOWER OIL

S = Saturated; M = monoenoic; D = dienoic fatty acid. Peaks are numbered from the start to the front. P = Palmitic; St = stearic; A = arachidic; B = bechenic; Li = lignoceric; O = oleic fatty acid.

TG Class	*Peak No.*	*ΔY (mm)*		*PN*		*TG groups*
		Calc.	*Found**	*Known*	*Found*	
S_2M	1	42.1	41.0 ± 2.8	X_1	56	LiPO + BStO + A_2O
	2	31.6	33.2 ± 2.0	X_2	54	AStO + BPO
	3	21.0	23.8 ± 2.4	52	52	St_2O
	4	10.4	12.6 ± 3.1	50	50	StPO
	5	−0.1	0	48	48	P_2O
SM_2	1	42.1	40.7 ± 2.8	X_1	56	LiO_2
	2	31.6	29.2 ± 2.0	X_2	54	BO_2
	3	21.0	20.7 ± 2.4	X_3	52	AO_2
	4	10.4	10.8 ± 3.1	50	50	StO_2
	5	−0.1	0	48	48	PO_2
S_2D	1	31.6	29.5 ± 2.0	X_1	54	A_2L + BStL
	2	21.0	20.5 ± 2.4	X_2	52	AStL + BPL
	3	10.4	9.6 ± 3.1	50	50	St_2L
	4	−0.1	0	48	48	StPL
	5	−10.7	−9.2 ± 1.6	46	46	P_2L
SMD	1	31.6	29.7 ± 2.0	X_1	54	LiOL
	2	21.0	23.7 ± 2.4	X_2	52	BOL
	3	10.4	10.5 ± 3.1	X_3	50	AOL
	4	−0.1	0	48	48	StOL
	5	−10.7	−12.7 ± 1.6	46	46	POL
D_2S	1	10.4	12.9 ± 3.1	X_1	50	BL_2
	2	−0.1	0	X_2	48	AL_2
	3	−10.7	−10.0 ± 1.6	46	46	StL_2
	4	−21.2	−21.9 ± 3.1	44	44	PL_2

* Mean + S.D., $n = 3$.

TABLE III

TRIGLYCERIDE ANALYSIS OF SUNFLOWER OIL

The TG classes and the TG groups are denoted as in Table II. tr. = trace.

TG class	*Silver nitrate TLC rel. %*	*TG group*	*RP-TLC rel. %**	*Total TG composition*
S_2M	0.2 ± 0.1	P_2O	24.8 ± 1.3	tr.
		StPO	39.2 ± 1.3	0.1
		St_2O	17.2 ± 1.5	tr.
		AStO + BPO	11.6 ± 1.0	tr.
		LiPO + BStO + A_2O	7.1 ± 1.3	tr.
SM_2	1.3 ± 0.5	PO_2	42.3 ± 1.3	0.5
		StO_2	37.5 ± 1.6	0.5
		AO_2	4.0 ± 1.1	tr.
		BO_2	12.6 ± 0.6	0.2
		LiO_2	3.8 ± 0.6	tr.
S_2D	1.3 ± 0.3	P_2L	27.3 ± 2.8	0.3
		StPL	46.7 ± 1.6	0.6
		St_2L	18.7 ± 2.1	0.2
		AStL + BPL	5.9 ± 1.2	0.1
		A_2L + BStL	4.2 ± 0.7	tr.
M_3	1.3 ± 0.3	O_3	–	1.3
SMD	9.0 ± 0.8	POL	51.1 ± 2.5	4.6
		StOL	38.0 ± 1.8	3.4
		AOL	2.7 ± 0.8	0.2
		BOL	6.0 ± 1.4	0.5
		LiOL	2.2 ± 0.6	0.2
M_2D	12.8 ± 0.2	LO_2	–	12.8
D_2S	16.6 ± 0.3	PL_2	48.5 ± 2.8	8.0
		StL_2	12.6 ± 2.6	2.1
		AL_2	4.7 ± 0.6	0.8
		BL_2	4.1 ± 0.9	0.7
D_2M	32.0 ± 0.5	L_2O	–	32.0
D_3	25.6 ± 0.7	L_3	–	25.6

* Mean ± S.D.

revealed more zones than expected according to Kaufmann and Wessels[6]. It has been found, however, that besides the basic saturated FAs (palmitic and stearic), sunflower oil may contain traces of other longer-chain members of this homologous series[7–11]. The GLC of the total FA composition of the sample confirmed the presence of arachidic and bechenic acids up to 1.5–2.0%, and traces of lignoceric acid. Table II presents the identification of the unknown TG groups. The D_2S class of the sample was of particular interest, since it presents a possibility to verify the identification. In this TG class, according to the literature, there are two TG groups, PL_2 and StL_2, with PN

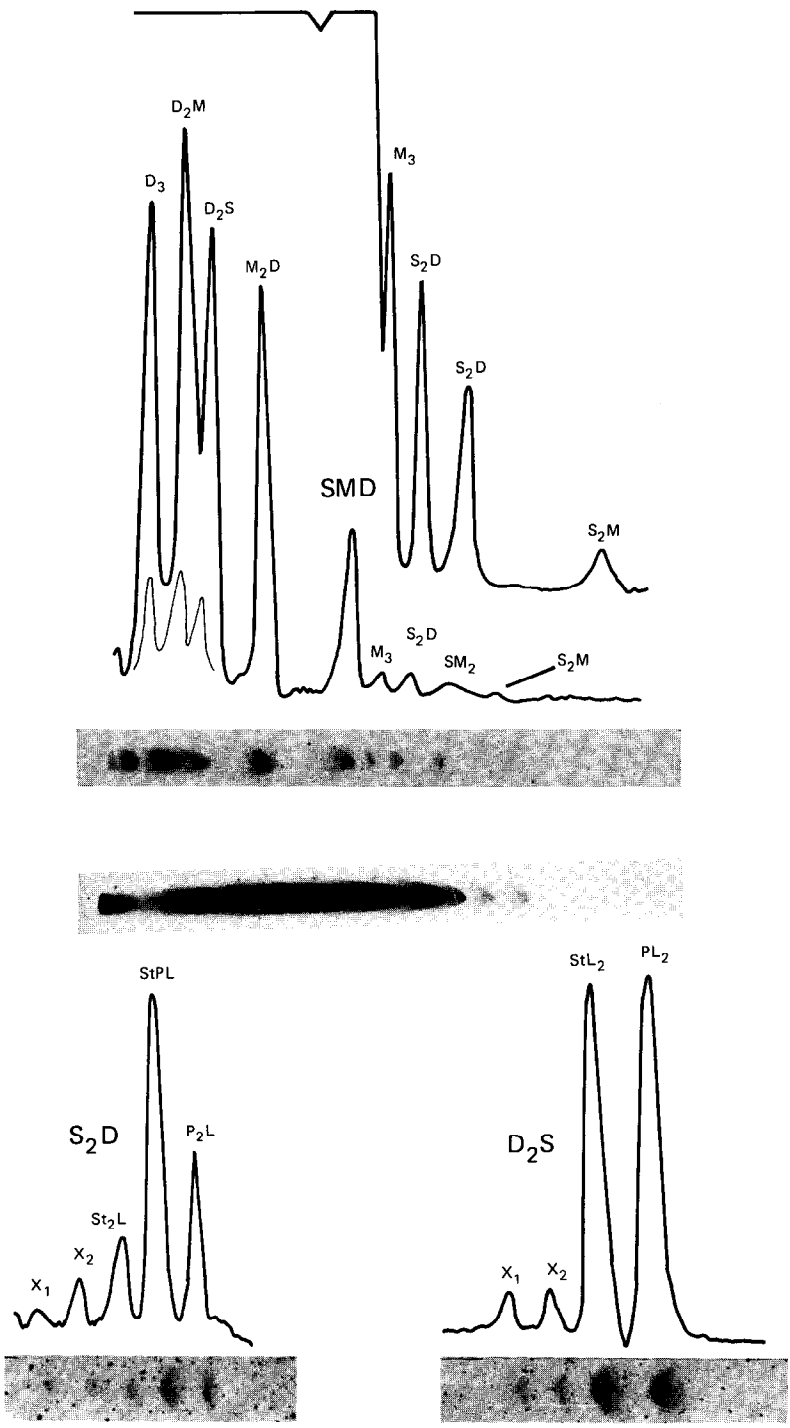
D2M
D3
D2S
M2D
M3
S2D
S2D
SMD
S2M
S2D
M3
SM2
S2M
StPL
S2D
P2L
St2L
X2
X1
StL2
PL2
D2S
X1
X2

Fig. 2

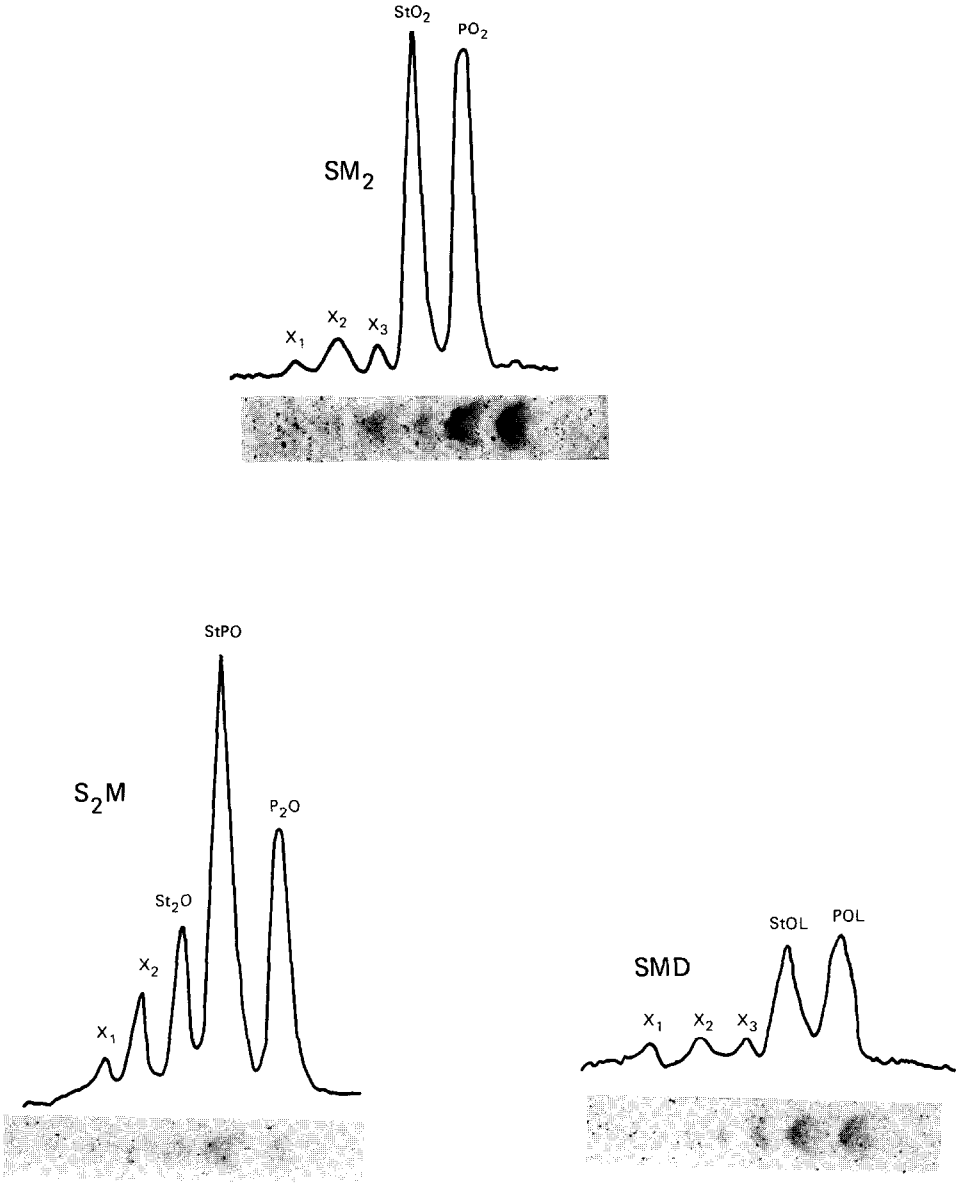

Fig. 2. Separation of a sunflower oil sample. Silver nitrate TLC of the total sample (p. 264, top). Conditions: 19 cm × 4 cm silica gel G plates, impregnated with 0.5% methanolic silver nitrate; mobile phase, 7 ml hexane–acetone (100:4.5, v/v). Reversed-phase TLC of the triglyceride classes. Conditions: 19 cm × 4 cm Kieselguhr G plates, silanization time 6 h; mobile phase, acetone–acetonitrile–water (70:30:12) (S_2M, S_2D), 70:30:14 (SM_2), (70:30:18) (SMD) and (70:30:20) (D_2S); development, ×2. The triglyceride classes and groups are as in Table II.

44 and 46 respectively. Assuming that the second peak in the densitogram pertains to the TG with PN = 48, we investigated how the two known TG groups are situated with respect to this TG. As seen from Table II, there is a good agreement between the calculated and the experimental ΔY values. Thus, concerning the PNs, all TG zones in the chromatograms are identified. Since the FA composition of the sample is known, it is easy to define the TG groups in the TG classes, which contain only one saturated FA. The zones with PN 52 and 54 in the S_2D class and those with PN 54 and 56 in the S_2M class are probably mixed. In Table II a TG group composition of these zones is assumed, which include all possible components. A further analysis by preparative RP-TLC[12] and GLC is necessary in order to determine the exact TG composition of these zones.

The separation of the sunflower oil sample by silver nitrate TLC and RP-TLC is shown in Fig. 2, where the respective chromatograms and densitograms are presented. The quantitative results are given in Table III. Twenty-eight TG groups have been determined, including those whose content in the sample is close to or even lower than 1%.

CONCLUSION

Very often a suggestion about the PNs of the components in a TG mixture can be made on the basis of the total FA composition and the TG class composition, obtained by silver nitrate TLC. In testing the mixture by RP-TLC in order to identify the components, the main problem is to define at least one component by its PN. The zone location on the plate, *i.e.*, its PN, can be determined by a simultaneous development with a suitable, easily available reference. In many cases this could be the O_3 class from olive oil, PN = 48. The rest of the TG components can be then identified by the use of the empirical relationship presented in this work. We consider this to be an useful way for obtaining the "fingerprint" of an unknown TG mixture.

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