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Analysis of mono-, di- and triglycerides in pharmaceutical excipients by capillary supercritical fluid chromatography*

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Abstract: Mono-, di- and triglycerides are important components of oils, fats and other natural products. Since in general fatty acids are mixtures and glycerol can be differently substituted, finger-prints of the composition are suitable for better characterization.

Since capillary supercritical fluid chromatography (SFC) employing carbon dioxide as mobile phase is compatible with flame ionization detection, it is possible to analyse many solutes at trace levels. Supercritical carbon dioxide offers higher solute diffusivity compared with the inert carrier gas conventionally used in gas chromatography and has a lower viscosity than the liquid solvents used in HPLC. Thus, glycerides of fatty acids can be separated and eluted at a lower temperature and with shorter analysis time in SFC. In this study the analysis of mono-, di- and triglyceride mixtures in several pharmaceutical excipients is reported using capillary SFC. Quantitative analysis is possible on the basis of a response factor established for each analyte. The accuracy of the method and its advantages are demonstrated.

Keywords: *Supercritical fluid chromatography (SFC); capillary SFC; excipients; glycerides; fats.*

Introduction

Natural oils are composed mainly of triglycerides of fatty acids and are currently used as excipients for oral liquid formulations. The synthetic or semi-synthetic derivatives of glycerol with saturated or unsaturated fatty acids are used in formulations as solubilizers, emulsifiers, emollients and stabilizers, whose properties are highly dependent on their composition.

Most pharmacopoeial monographs specify methods of analysis such as saponification value, iodine value, hydroxyl value and melting point. Gas chromatography (GC) is commonly used for the assay of fatty acids composition. Monoglycerides may be determined by titration. GPC may be used for the global determination of monoglycerides, diglycerides or triglycerides, but the exact composition is not obtained by this method.

These methods are not sufficient for a full characterization of the excipients. Since capillary supercritical fluid chromatography (SFC) employing carbon dioxide as mobile phase is compatible with flame ionization detection, it is possible to analyse solutes at trace levels. Supercritical carbon dioxide offers higher solute diffusivity compared with the inert carrier gas used in GC and has a lower viscosity than the liquid solvents used in HPLC. Thus glycerides can be separated and eluted at a lower temperature and with shorter analysis time.

SFC has been already proposed [1–4] for glycerides. A capillary SFC method using a SB-Octyl column has been developed. It allows the separation and determination of the mono-, di- and triglycerides of fatty acids from C6 to C22 in less than 1 h. The method has been applied to different excipients.

Experimental

Equipment and methods

The SFC apparatus was a Lee Scientific Series 600 SFC instrument (Lee Scientific, Salt Lake City, UT, USA) equipped with a flame ionization detector (FID). The flame ionization detector was kept at 375°C. The pump was cooled by circulating a water–ethylene glycol mixture at 7°C using a Haake refrigerating unit (Haake, Karlsruhe, Germany).

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Carbon dioxide (Scott Speciality Gases, Plumsteadville, USA) was used as mobile phase. The sample was introduced using a Rheodyne model 7520 injector (Rheodyne, Cotati, CA, USA). Commercially available sample rotor with a 0.2-μl loop was used. A 10 m × 50 μm i.d. SB-Octyl 50 open tubular capillary column (Lee Scientific) with a film thickness of 0.25 μm was used as analytical column. A frit restrictor (25 cm × 50 μm i.d., Lee Scientific) was used to maintain the pressure. Data collection and reporting were performed on a Perkin–Elmer Class 2000 system.

Samples were introduced by direct injection onto the analytical column. The density was kept at a low value (0.19 g ml^{-1}, oven temperature 90°C, pressure approximately 94 bars) during injection. After 6 min hold, the density was programmed to 0.40 g ml^{-1} at a rate of 0.04 g ml min^{-1}, then after 4 min hold at d = 0.40 g ml^{-1} the density was programmed to 0.80 g ml^{-1} at a rate of 0.01 g ml^{-1} min^{-1} (oven temperature 90°C).

Materials

Standard solutions. Approximately 10 mg of each standard glyceride were dissolved in 10 ml dichloromethane.

Derivatives of caprylic and capric acids. Approximately 140 mg of the excipients were dissolved in 20 ml dichloromethane.

Derivatives of myristic acid, palmitic and stearic acids. Approximately 30 mg of the excipients were dissolved in 20 ml dichloromethane.

Derivatives of oleic acid. Approximately 100 mg of the excipients were dissolved in 20 ml dichloromethane.

Determination of detector response factor and quantitation of glycerides in the analysed samples [5]

In a sample, peak area values are related to the concentration of the respective component through a proportionality factor, f_i, depending mainly on the chemical nature of the substance and on the type of detector used.

These factors can be expressed in units per mass relatively to a given standard:

$$f_i = (A_{st}m_i/A_i m_{st})f_{st}, \quad (1)$$

but in analytical practice (GC analysis) relative molar response (RMR_i) are more appropriate

$$RMR_i = (A_i n_{st}/A_{st} n_i)RMR_{st}. \quad (2)$$

The factors f_i are so defined that they are inversely proportional to RMR_i:

$$f_i = M_i RMR_{st}/M_{st} RMR_i, \quad \text{with } f_{st} = 1. \quad (3)$$

In the analysed sample, these f_i (respectively RMR_i) are used to help in the calculation of the true concentration value from the peak areas obtained in the chromatogram. For each component the individual part c_i is defined as

$$c_i\,(\%) = 100 \times f_i A_i/\Sigma f_i A_i, \quad \text{with } f_i,$$
$$c_i\,(\%) = 100 \times (M_i A_i/RMR_i)/(\Sigma M_i A_i/RMR_i), \quad \text{with } RMR_i. \quad (4)$$

In this study, monopalmitin was taken as the standard. For a FID it has been established that $RMR_i = n_i \times 100$, n_i representing the number of C-atoms, e.g. the number of CH_2-groups in the molecule.

A RMR value of 1800 was assumed to monopalmitin and eight calibration standard mixtures containing known concentrations of standard mono-, di-, triglycerides (Sigma) from C6 to C22 were used for the determination of the RMR of each analyte. These RMR factors were calculated accordingly to the value assumed to monopalmitin (see equation 2). The abbreviations used above are: A, peak area; i, component; m, mass; M, molecular weight; n, mole number; RMR, relative molar response; st, standard.

Selectivity, accuracy and reproducibility of the method

The SB-Octyl 50 column is able to separate 1,2 glyceride isomers from the 1,3 isomers and also triglycerides which just differ in the number of double bonds (Table 1; Fig. 1). Glycerol is not eluted. Pure fatty acids are eluted at the beginning but the peak shape is not adequate.

In a class (e.g. monoglyceride) solutes elute in the order of increasing molecular weight (Fig. 2).

The accuracy of the method can be demonstrated by comparing actually found and theoretically expected amounts for the 20 individual glycerides of two standard mixtures. The amounts calculated according to the pro-

Table 1

	Standards	Retention time (min)*
1	Monocaprylin (C8)	11.63
2	1,2-Dicaproin (C6)	12.68
3	1,3-Dicaproin	12.82
4	Monocaprin (C10)	12.95
5	Monolaurin (C12)	14.15
6	Tricaproin	14.50
7	1,3-Dicaprylin	15.21
8	Monomyristin (C14)	15.76
9	Monopalmitin (C16)	17.41
10	1,2-Dicaprin	17.94
11	1,3-Dicaprin	18.33
12	Monolinolenin (C18:3)	18.40
13	Monolinolein (C18:2)	18.51
14	Tricaprylin	18.51
15	Monoolein (C18:1)	18.77
16	Monostearin (C18:0)	19.31
17	Monoarachidin (C20)	21.26
18	1,2-Dilaurin	21.32
19	1,3-Dilaurin	21.76
20	Tricaprin	23.44
21	1,2-Dimyristin	24.93
22	1,3-Dimyristin	25.36
23	1,3-Dipentadecanoin	27.17
24	Trilaurin	28.12
25	1,3-Dipalmitin	28.80
26	1,3-Dilinolenin or tritridecanoin	30.43
27	1,3-Dilinolein	30.57
28	1-Palmitoyl-3-stearoyl rac glycerol	30.59
29	1,3-Diolein	31.07
30	1,3-Distearin	32.07
31	Trimyristin	32.52
32	1,2-Diarachidin	34.61
33	Tripentadecanoin	34.71
34	1,3-Diarachidin	35.11
35	Tripalmitin	36.73
36	1,2-Dipalmitoyl-3-oleoyl rac glycerol	37.60
37	Trilinolenin	38.18
38	1,2-Dioleoyl-3-palmitoyl rac glycerol	38.44
39	Trilinolein	38.58
40	Triheptadecanoin	38.72
41	1-Palmitoyl-2-oleoyl-3-stearoyl rac glycerol	38.90
42	Triolein	39.29
43	1,2-Distearoyl-3-palmitoyl rac glycerol	39.32
44	1,2-Distearoyl-3-oleoyl rac glycerol	40.13
45	Tristearin	40.58
46	Trinonadecanoin	42.35
47	Triarachidin	44.00
48	Tribehenin (C22)	47.18

* Experimental conditions as given in the text.

cedure described above are in good agreement with the theoretically expected amounts, e.g. differences 0–4% relatively for all standard glycerides except monoolein, monostearin and monolinolein where differences amount to 5–8% (Tables 2 and 3).

A comparison of the composition in mono-, di and triglycerides obtained by SFC and GPC given in Table 4 for a standard mixture demonstrates a very good agreement.

A relative standard deviation (RSD) of the absolute peak area of 1.13% was observed for seven individual injections of the same glyceryl monomyristate solution. This is well within the range reported in the literature for this injection method.

Results

The SFC chromatograms and the corresponding compositions for the analysis of different excipients are given in Figs 3–8 and Tables 5–12. The composition of fatty acids and the mono-, di- and triglycerides content are calculated for comparison purpose. The results are compared with the manufacturer

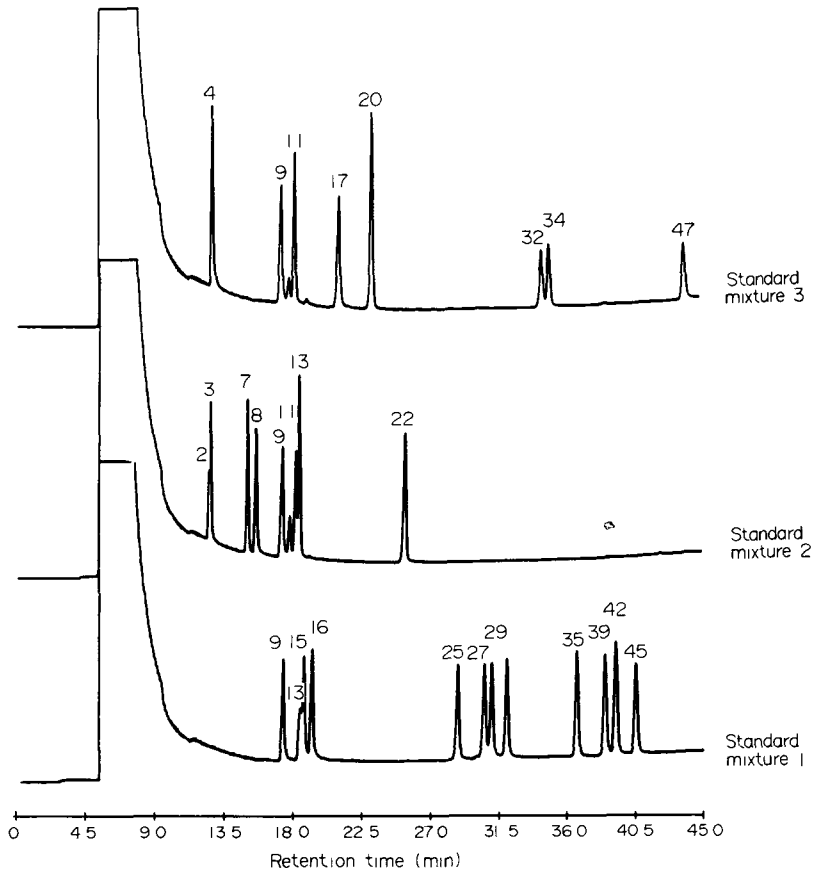

Figure 1
Chromatograms obtained for standard mixtures. Experimental conditions as in the text.

data or with the analysis of fatty acids carried out by GC and the mono-, di- and triglyceride contents determined by GPC.

Derivatives of caprylic and capric acids (C8/C10)

Medium chain triglycerides (DAB9, Miglyol 812; Fig. 3; Table 5). All peaks could be identified. The standards of mixed di- and triglycerides (C8–10, C8–10–10, C8–8–10) being not available, the corresponding peaks have been identified by their retention.

No monoglycerides were detected, but the sample also contained diglycerides. The composition of fatty acids calculated by addition of the different peak areas is in good agreement with the typical GC composition, namely: C8, 61%; and C10, 39% with SFC; compared with the typical composition: C8, 50–65%; C10, 30–45%; as stated by the manufacturer.

Mono, di- and triglycerides of caprylic and capric acids (Imwitor 742; Fig. 4; Table 6). Two batches have been analysed. The main difference is the presence of glyceryl monopalmitate (C16) or an unknown component in one batch. Comparison of the SFC results with the GC values determined for batch 2 shows good agreement, viz. GC: C8, 56%; C10, 36%; others, 7.8%. SFC: C8, 60%; C10, 37%; others, 3%. The manufacturer declares a

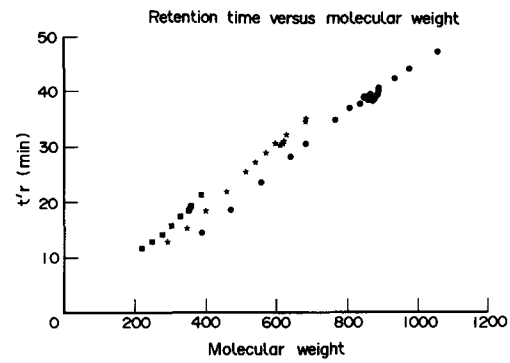

Figure 2
Retention time vs glyceride molecular weight. ■, Monoglyceride; ★, diglyceride; ●, triglyceride. Experimental conditions as stated in the text.

Table 2
Accuracy of the SFC method: example with standard mixture 1

Component	Molecular weight	RMR-factor	Theoretical % (mass weighed)	Experimental % obtained
Monopalmitin C16	330.5	1800	8.13	8.08
Monolinolein C18:2	354.5	1233	8.70	9.42
Monoolein C18:1	356.5	1836	8.13	7.66
Monostearin C18	358.5	2514	7.72	7.13
1,3-Dipalmitin	568.9	3714	7.80	7.54
1,3-Dilinolein	617.0	3888	8.78	9.00
1,3-Diolein	620.9	3743	8.54	8.64
1,3-Distearin	624.9	4163	8.29	8.40
Tripalmitin	807.3	5399	8.46	8.36
Trilinolein	879.4	5867	8.46	8.52
Triolein	885.4	6235	8.86	8.89
Tristearin	891.5	5718	8.13	8.36

Table 3
Accuracy of the SFC method: example with standard mixture 2

Component	Molecular weight	RMR-factor	Theoretical % (mass weighed)	Experimental % obtained
1,2-Dicaproin	288.4	1025	7.68	7.50
1,3-Dicaproin	288.4	2033	7.68	7.41
1,3-Dicaprylin	344.5	1992	13.97	14.01
Momyristin	302.5	1701	13.69	13.58
Monopalmitin	330.5	1800	14.67	14.71
1,2-Dicaprin	400.6	1535	6.985	6.92
1,3-Dicaprin	400.6	3713	6.985	6.97
Tricaprylin	470.7	3111	14.10	14.38
1,3-Dimyristin	512.8	3423	14.24	14.52

Table 4
Comparison of SFC and GPC methods for a standard of given composition

	SFC	GPC	% Mass (theory)
Monoglycerides (C16, C18, C18:1, C18:2)	42.49	43.28	43.42
Diglycerides (C16, C18:1, C18:2)	33.62	32.58	33.36
Triglycerides (C18:1, C18:2)	23.89	24.14	23.22

Table 5
Composition of medium chain triglyceride (Miglyol 812)

Component:	C8–8	C8–10	C8–8–8	C8–8–10	C8–10–10	C10–10–10
Amount:	0.30%	1.5%	22.8%	43.0%	26.6%	5.8%

ΣCaprylic (C8): 61%. ΣCapric (C10): 39%.

monoglycerides content of 40–50% which corresponds well with the SFC values of 50% for batch 1 and 47% for batch 2.

Derivatives of myristic acid

Glyceryl trimyristate (Fig. 5; Table 7). Two batches of two different manufacturers were analysed. The experimental results are in good agreement with those declared by the manufacturers. The batches contain 93–94% glyceryl trimyristate, 95–96% triglycerides, 4–5% diglycerides and no monoglyceride. The fatty acid compositions are in agreement with the GC values determined for batch 2. The second manufacturer's batch contains oleic acid and therefore could be more sensitive to oxidation.

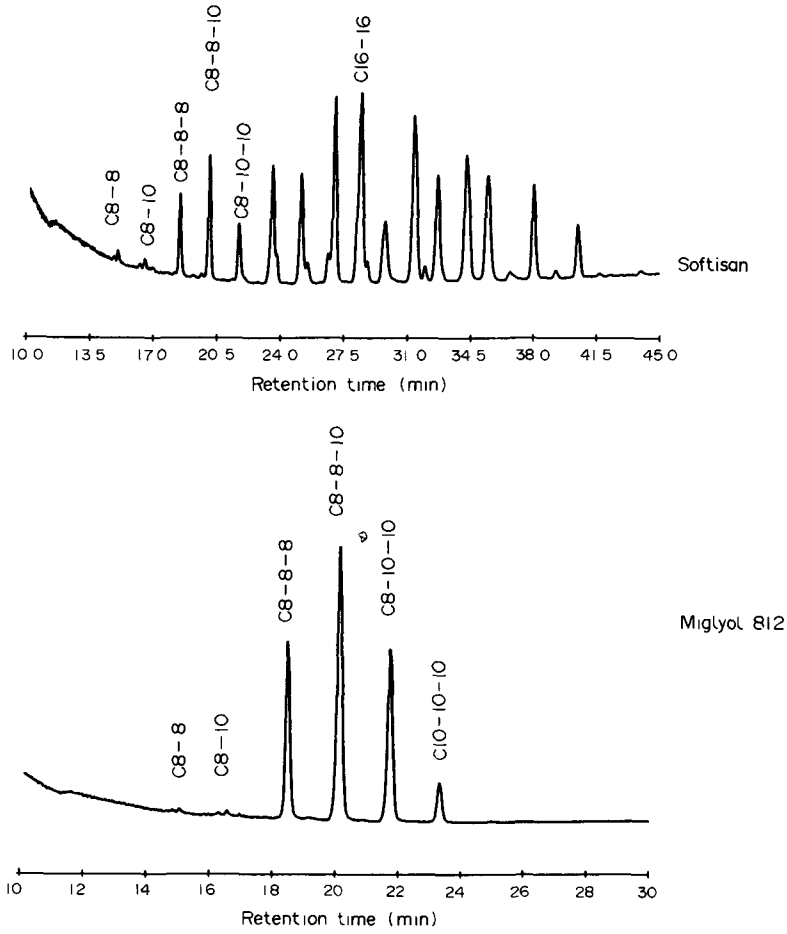

Figure 3
Derivatives of caprylic and capric acids (Miglyol 812 and softisan). Experimental conditions as stated in the text.

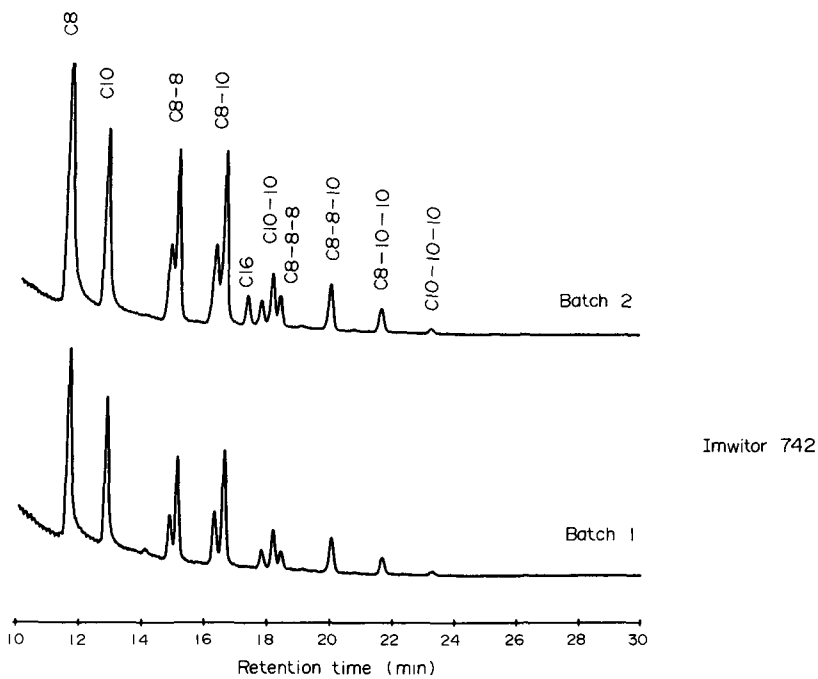

Figure 4
Derivatives of caprylic and capric acids (Imwitor 742). Experimental conditions as stated in the text.

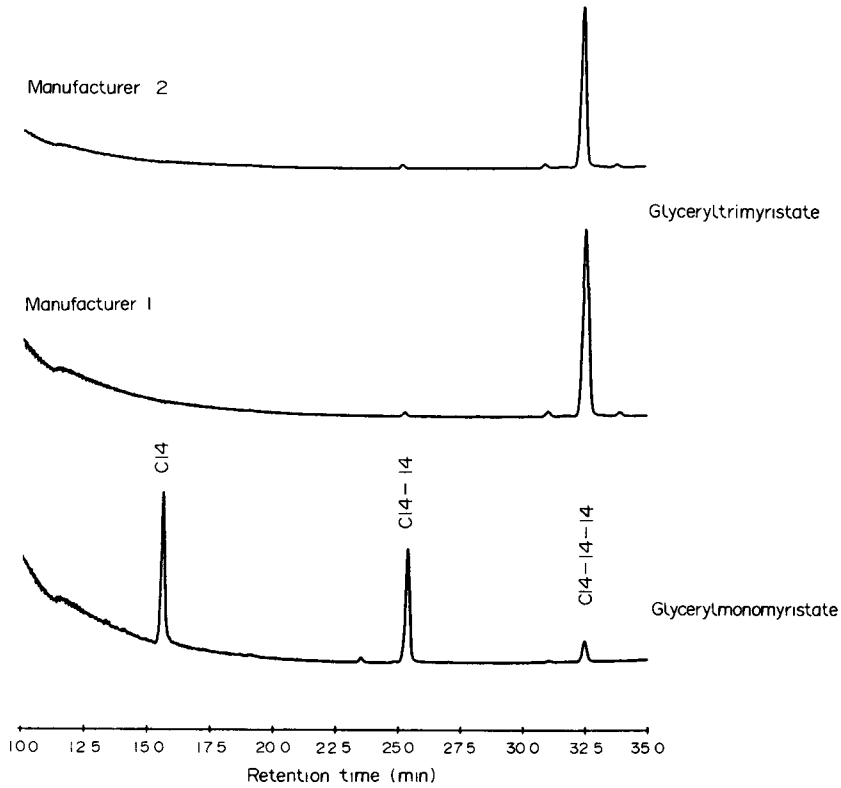

Figure 5
Derivatives of myristic acid. Experimental conditions as stated in the text.

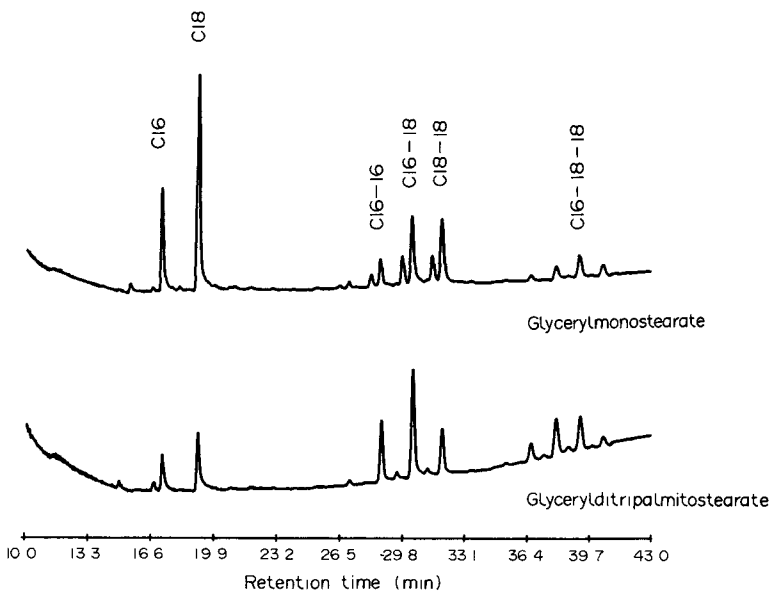

Figure 6
Derivatives of stearic acid and palmitostearic acids. Experimental conditions as stated in the text.

Table 6
Composition of mono-, di- and triglycerides of caprylic and capric acids

Component:	C8	C10	C12	C16	C8–8	C8–10	C10–10	C8–8–8	C8–8–10	C8–10–10	C10–10–10
Batch 1	27.7%	22.3%	0.1%		15.9%	19.0%	6.0%	2.0%	4.7%	2.25%	0.1%
Batch 2	27.9%	19.2%	—	2.4%	18.0%	19.5%	5.4%	2.0%	3.2%	1.9%	0.5%

Batch 1: Σcaprylic, 59%; Σcapric, 41%. Batch 2: Σcaprylic, 60.5%; Σcapric, 37%; Σpalm, 2.5%.

Table 7
Composition of glyceryl trimyristate

Component:	1,3-Dimyr. C14	Dioleic C18–18	Other di.	Trimyristin	Other tri.
Manuf. 1	1.5%		3%	92.8%	2.7%
Manuf. 2	1.8%	2.7%		93.7%	1.8%

Manuf. 1: Σtri, 95.4%. Manuf. 2: Σtri, 95.5%; no monoglycerides. Manuf. 2: Σmyristic, 95.4%; others, 4.6%. GC: myristic, 98.1%; palm. 1%.

Table 8
Composition of glyceryl monomyristate

Component:	Monomyr. C14	Tricaprin C10	1,3-Dimyrist.	Trimyristin C14
Amount:	53.7%	1.2%	37%	8.1%

Table 9
Composition of glyceryl monostearate

Component:	C16	C18	C16–16	C16–18	C18–18	Tri C16	C16–16–18	C18–18–16	Tri C18
Amount:	16.5%	32.1%	6.3%	17.6%	16.2%	1.0%	3.6%	4.6%	2.1%

SFC: Σmono, 48.6%; Σdi, 40.1%; Σtri, 11.3%. GPC: Σmono, 48.7%; Σdi, 41.2%; Σtri, 10.1%.

Table 10
Composition of glyceryl di-, tripalmitostearate

Component:	C16	C18	C16–16	C16–18	C18–18	Tri C16	C16–16–18	C18–18–16	Tri C18
Amount:	10.8%	13.2%	13.1%	25.6%	10.9%	5.2%	9.5%	9.0%	2.7%

SFC: Σmono, 24.0%; Σdi, 49.6%; Σtri, 26.4%. SFC: ΣC16, 51.3%; ΣC18, 48.7%. GPC: Σmono, 27.2%; Σdi, 52.1%; Σtri, 20.7%. GC: ΣC16, 44%; ΣC18, 48%; Σother, 8%.

Table 11
Composition of glyceryl mono, -dioleate

Component:	Monopalm	Monool.	Mono.	Dilinol.	Diolein	Other di	Triolein	Other tri
Tegin O	2.8%	54.5%	6.8%	8.0%	17.0%	8.7%	2.2%	—
GMO-33	4.0%	41.0%	4.0%	9.5%	21.5%	12.5%	5%	2.5%

SFC: Tegin O: Σmono, 64%; Σdi, 34%; Σtri, 2%. SFC: GMO-33: Σmono, 49%; Σdi, 44%; Σtri, 7%. GPC: Tegin O: Σmono, 61%; Σdi, 34%; Σtri, 5%. GPC: GMO-33: Σmono, 47%; Σdi, 43%; Σtri, 10%.

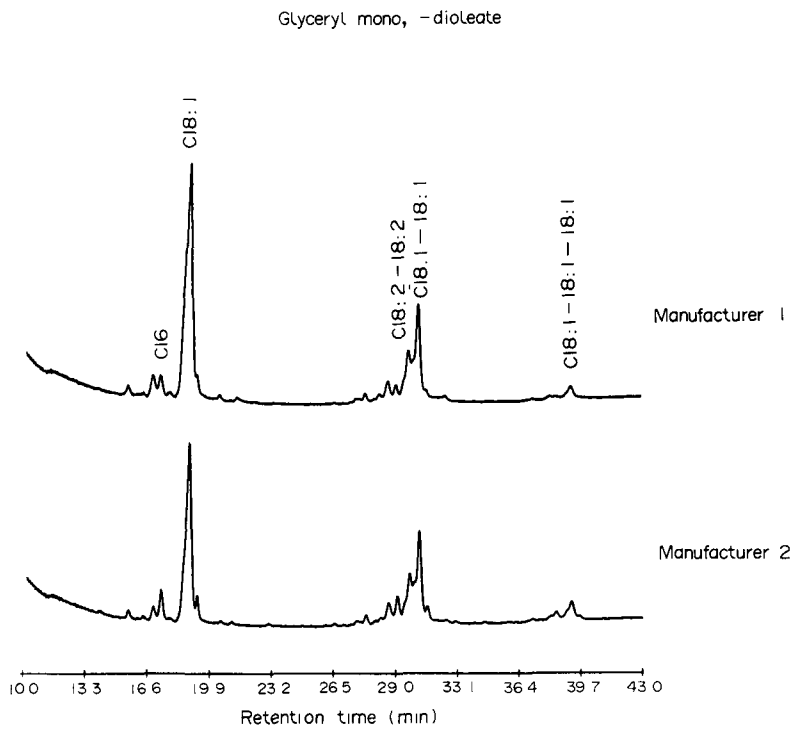

Figure 7
Derivatives of oleic acids: glyceryl mono, -dioleate. Manufacturer 1, Tegin O; Manufacturer 2, GMO-33. Experimental conditions as stated in the text.

Glyceryl monomyristate (Fig. 5; Table 8). Ninety-nine per cent of the fatty acid content is myristic acid. Only 54% of glyceryl monomyristate is found, which corresponds to the typical value of 50% given by the manufacturer. A better description of the excipient would be 'mono, di- and triglycerides of myristic acid'.

Derivatives of palmitic and stearic acids

Glyceryl monostearate (Fig. 6; Table 9). The same ratio of the acids is found in mono-, di- and triglycerides. The SFC values give 48.6% monoglycerides, 40.1% diglycerides and 11.3% triglycerides. These values are in very good agreement with the GPC values (respectively, 48.7, 41.2 and 10.1%). A better declaration would be 'mono-, di- and triglycerides of stearic acid'.

Glyceryl ditripalmitostearate (Fig. 6; Table 10). Good agreement is found between SFC and GC for the fatty acids composition and between SFC and GPC for mono-, di- and triglycerides content. The content found in monoglycerides is higher (24% SFC and 27.2% GPC) than the value obtained by titration (12%). The main part is the diglycerides: 49.6%.

Derivatives of oleic acid

Glyceryl mono, -dioleate (Fig. 7; Table 11). Two samples of two different manufacturers (Tegin O and GMO33) were analysed.

Table 12
Composition of natural oils

Component:	Diglycerides	1,2-Diol. 3-palm.	Triolein	Trilinolein	Other tri
Corn oil 1	2.3%	—	*	23.7%	73.9%
Corn oil 2	4.2%	—	*	22.1%	73.7%
Olive oil	2.0%	26.0%	60.7%	—	11.3%

*In corn oil triolein is determined with other mixed triglycerides.

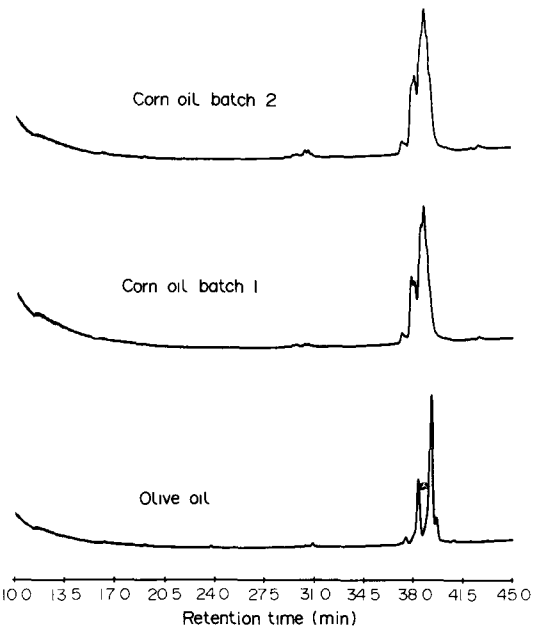

Figure 8
Natural oils. Experimental conditions as stated in the text.

Although GMO-33 is declared to be monooleate, the same profile as Tegin O is obtained. Tegin O contains a bit more monoglyceride. The mono-, di- and triglycerides contents obtained by SFC are in good agreement with GPC values.

Natural oils (Fig. 8; Table 12). Corn oil and olive oil are mainly triglycerides (96–98%). For corn oil triolein is not well separated from other mixed triglycerides.

Softisan. The excipient is declared as a derivative of capryl, capric and stearic acids. The SFC chromatogram shows a considerable number of peaks, all of which could not be identified.

Conclusions

The selectivity and accuracy of the SFC method for the analysis of glyceride standard mixtures are clearly demonstrated. Comparison of the information given by GPC for mono-, di- and triglycerides content and by GC for the fatty acid composition of different excipients shows good agreement. The avantage of the use of SFC for the analysis of glyceride derivatives is clearly demonstrated by several examples: a more detailed knowledge of the excipient may be obtained in a very short time; furthermore, the described method is easy to apply.

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