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Note

Evaluation of selected stationary phases suitable for the gas–liquid chromatographic analysis of triglycerides

R. ANEJA, A. BHATI, R. J. HAMILTON, F. B. PADLEY and D. A. STEVEN*

Department of Chemistry, Liverpool Polytechnic, Liverpool (Great Britain) and Unilever Research Laboratory, Colworth (Great Britain)

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High-temperature gas–liquid chromatography (GLC) is an established technique for the separation of triglycerides according to molecular weight[1–4]. Because of the low vapour pressure of triglycerides, present methods are based upon the use of short columns of low stationary phase loadings and using high temperatures. Excellent reviews by Litchfield[5] and earlier by Pierce[6] optimise conditions relating to the quantitative analysis of triglyceride mixtures.

Many compounds have been reported as suitable for use as stationary phases in GLC, but little information is available as to their applicability to triglyceride analysis. Polyesters of succinic acid, a cyanopropyl phenyl siloxane[7] (Silar 5CP) and more recently a polar siloxane[8] (Silar 10C) have been used for the resolution of saturated and unsaturated diacylglycerols. Unfortunately, they have only moderate thermal stability and similar separations using triglycerides cannot be satisfactorily effected. Silar 10C showed some resolution of triglycerides by degree of unsaturation, but the columns could only be used for a few months at 250–270°.

The major criterion in selecting a stationary phase suitable for triglyceride analysis is its thermal stability with only those which can operate at temperatures of 325° or greater being considered suitable. Hence OV-1, OV-101, OV-17, OV-22, SE-30, SE-52, JXR, Dexsil 300 and PmPE have been the stationary phases of choice, but all tend to be non-polar compared to the polyesters which are used in fatty acid analysis.

In the present study, selected stationary phases were evaluated as to their resolution and quantitative recovery of model monoacid triglyceride mixtures, consisting of saturated and unsaturated triglycerides. The stationary phases evaluated were OV-17, OV-22, Dexsil 300GC, PmPE (high polymer) and a polyphenyl ether sulphone phase (Poly-S-179). The analyses enabled the phases to be placed in order of their selectivity to quantitatively resolve triglyceride mixtures. The upper thermal limits for the stationary phases are given in Table I.

* To whom correspondence should be addressed at the following address: Department of Chemistry, Liverpool Polytechnic, Liverpool, Great Britain.

TABLE I

RECOMMENDED UPPER THERMAL LIMITS FOR THE STATIONARY PHASES

Stationary phase	*Type of phase*	*Recommended thermal limit*
OV-17	50% methyl phenyl silicone	375°
OV-22	35% methyl phenyl silicone	350°
Dexsil 300GC	carborane methyl silicone	500°
PmPE (high polymer)	polyphenyl ether	450°
Poly-S-179	polyphenyl ether sulphone	400°

MATERIALS AND METHODS

Materials

Dexsil 300GC, OV-17 and OV-22 were purchased from Phase Separations (Queensferry, Great Britain). PmPE (high polymer) was purchased from Varian Aerograph, Walnut Creek, Calif., U.S.A.. Poly-S-179 was purchased as a 2% (w/w) column packing on Supelcoport (100–120 mesh) from Supelco (Bellefonte, Pa., U.S.A.). Supelcoport (100–120 mesh) was purchased from Supelco (chemically treated to reduce adsorption effects). Trilaurin, trimyristin, tripalmitin, tristearin, triolein and trilinolein (all 99% purity) were purchased from Sigma (St. Louis, Mo., U.S.A.).

Gas–liquid chromatography

A Pye Unicam Model 104 gas chromatograph equipped with flame ionisation detectors was used for the analyses.

Column packings were prepared by coating the stationary phases onto the solid support, from chloroform solution, by the solvent evaporation technique[9]. Glass columns 0.45 m × 2.5 mm I.D. were filled with the column packings with the aid of a vacuum pump and a vibrator to ensure closely packed columns. The columns were satisfactorily conditioned by heating at 350° for 8 h with a nitrogen flow-rate of 80 ml/min.

The injection port temperature and detector were maintained at 350°. A reference saturated triglyceride mixture was prepared (see Table II) and analysed on each column. A temperature program in the range 220–350° at 3–4°/min with a nitrogen flow-rate of 80 ml/min was used to elute the triglycerides.

Evaluation methods

(a) The recovery of a model saturated monoacid triglyceride mixture (Table II)

TABLE II

COMPOSITION OF THE SATURATED MONOACID TRIGLYCERIDE MIXTURE

An aliquot of 5 μl was used for the analyses (= 34.7 μg) to determine response factors.

Triglyceride	*Weight (g) taken to 100 cm³*	*Weight %*	*Mole %*
Trilaurin	0.1763	25.39	29.92
Trimyristin	0.1728	24.91	25.94
Tripalmitin	0.1708	24.62	22.96
Tristearin	0.1740	25.08	21.18

was evaluated by determining the weight and molar response factors for the eluted triglycerides. Peak areas were measured by triangulation, quantitative weight response factors (F_w) and molar response factora (F_m) for individual triglycerides were calculated by the internal normalisation technique. Here F_w = actual wt.%/area % and F_m = actual mole%/area %. A value of 1.00 was assigned to F_w and F_m for trilaurin (primary standard) which was assumed to be completely recovered from the column. The primary standard was then included in all calibration mixtures so that the calibration factors from all GLC runs were comparable.

(b) The resolution of the triglyceride peaks was measured by determining the separation factor ΔC (ref. 10), defined as the minimum carbon number difference between two adjacent triglycerides that can be separated with baseline resolution (see Fig. 1).

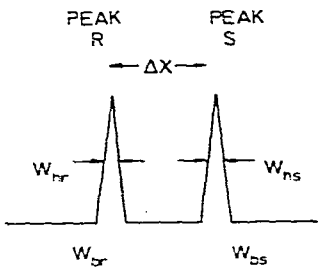

Fig. 1. Calculation of separation factor (ΔC). Any two triglyceride peaks R and S with a difference in carbon number may be chosen. The average baseline width ($W_{b\,rs}$) of triglyceride peaks in this region was found using the relationship $W_{b\,rs} = 1.6\,(W_{hr} + W_{hs})$. Then the distance ($\Delta X$) between the apices of the two peaks was measured. The maximum number of peaks (M) which can be separated with baseline resolution is: $M = \Delta X/W_{b\,rs} = \Delta t/1.6\,(W_{hr} + W_{hs})$. The minimum carbon number difference which can be resolved with baseline resolution (ΔC) is $6/M$, therefore $\Delta C = 9.6\,(W_{hr} + W_{hs})/\Delta X$.

(c) The resolution of saturated and unsaturated triglycerides of the same carbon number was determined by analysing a reference mixture of tristearin, triolein and trilinolein. Response factors for triolein and trilinolein were only calculated for stationary phases that resolved the reference mixture.

RESULTS AND DISCUSSION

The response of the flame ionisation detector is known to vary with load levels[11]. In the present study, no attempt was made to ascertain this variation or to correct for it. Instead, a constant weight of the reference triglyceride mixture was used to evaluate the stationary phases. The response factors given in Table III relate the actual composition of the test mixture to the area response recorded on the chromatogram. These response factors give an accurate indication of sample recovery.

On short columns, (0.45–0.60 m) OV-17, OV-22, Dexsil 300GC and PmPE separate triglycerides only on the basis of molecular weight (carbon number). Good resolution, combined with good recovery, is obtained for most of these stationary phases in an acceptable analysis time of less than 45 min. However, PmPE gave unacceptably high response factors, indicating large losses of the higher molecular weight triglycerides, and is therefore unsuitable for use in the quantitative analysis

TABLE III

EXPERIMENTALLY DETERMINED WEIGHT AND MOLAR RESPONSE FACTORS ON 2% LOADINGS OF THE STATIONARY PHASES IN 0.45 m × 2.5 mm I.D. GLASS COLUMNS

Stationary phase	*Weight calibration factors (F_w)*						*Molar calibration factors (F_m)*					
	*36***	*42*	*48*	*54*	*$54^{3=}$****	*$54^{6=}$*	*36*	*42*	*48*	*54*	*$54^{3=}$*	*$54^{6=}$*
Theoretical*	1.00	0.97	0.95	0.93								
OV-17	1.00	0.99	1.08	1.24			1.00	0.88	0.85	0.93		
OV-22	1.00	0.99	1.11	1.26			1.00	0.87	0.87	0.92		
Dexsil 300GC	1.00	1.01	1.24	1.55			1.00	0.89	0.97	1.10		
PmPE	1.00	1.25	1.97	4.17			1.00	1.12	1.56	3.00		
Poly-S-179	1.00	0.78	0.85	1.24	1.70	2.63	1.00	0.89	0.88	1.14	1.22	1.90

* Theoretical F_w response factors for specific triglycerides, calculated by assuming that all of the injected sample reaches the detector and that the flame ionisation detector response is proportional to the hydrocarbon content of each triglyceride.

** Total number of carbon atoms in the fatty acid acyl groups of each triglyceride, referred to as the carbon number.

*** Refers to the total number of alkenic linkages in each triglyceride. Therefore, for monoacid triglycerides, $54^{3=}$ is triolein.

of triglyceride mixtures. Losses could be due to pyrolysis during vapourisation of the sample, on-column degradation, adsorption or condensation. Since the experimental conditions were the same for all of the stationary phases, then adsorption of triglycerides onto the stationary phase is the most likely cause of sample loss. No low-molecular-weight fragments could be detected which would indicate pyrolysis or degradation.

The ΔC values are a more practical guide than the number of theoretical plates in determining if a specific column would yield a desired separation. Litchfield *et al.*[10] defined an empirical ΔC value equal to the minimum carbon number difference between two triglycerides that could be separated with baseline resolution in the C_{42-48} region of the chromatogram. In the present study, ΔC values in both the C_{42-48} and C_{48-54} regions of the chromatogram were calculated since most natural fats contain primarily C_{48}, C_{50}, C_{52} and C_{54} triglycerides. A linear relationship exists between retention time and the number of carbon atoms in a homologous series of compounds, for temperature programmed operation. However, because of a continuously decreasing difference in volatility between higher molecular weight pairs of

TABLE IV

EXPERIMENTALLY DETERMINED SEPARATION FACTORS (ΔC VALUES) IN THE C_{42-48} AND C_{48-54} REGIONS OF THE CHROMATOGRAM

All the stationary phases were used as 2% (w/w) coatings on Supelcoport (100–120 mesh).

Stationary phase	*ΔC_{42-48}*	*ΔC_{48-54}*
OV-17	1.9	2.1
OV-22	2.2	2.6
Dexsil 300GC	1.9	2.1
PmPE	1.9	2.1
Poly-S-179	2.1	2.2

triglycerides, they are eluted at progressively closer intervals and so the linear relationship exists only over a limited range of the triglyceride series. This is shown by an increase in the ΔC_{48-54} value compared to the ΔC_{42-48} value for all of the stationary phases evaluated. The results are given in Table IV. The low separation factors obtained on the OV-17 column, combined with almost quantitative recovery of the sample, make it one of the stationary phase of choice when resolving and quantitatively analysing triglyceride mixtures on the basis of molecular weight.

For all of the stationary phases a loading of 2% (w/w) was found to be opti-

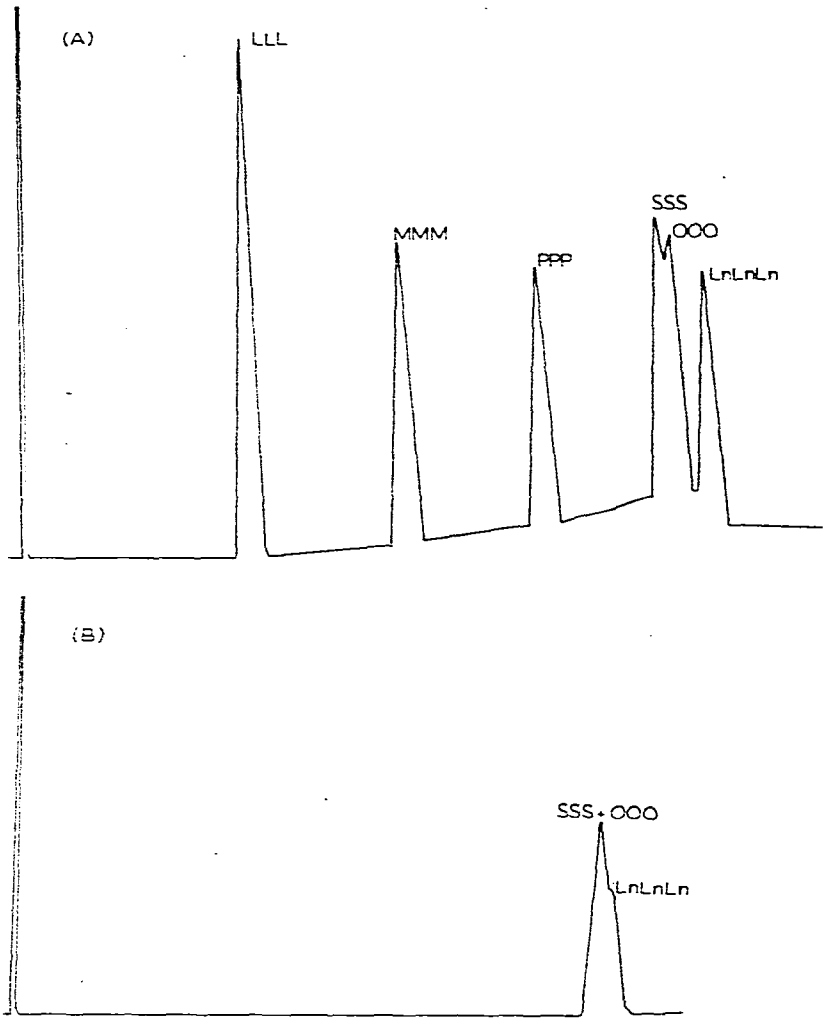

Fig. 2. (A) Chromatogram of a synthetic mixture of trilaurin (LLL), trimyristin (MMM), tripalmitin (PPP), tristearin (SSS), triolein (OOO) and trilinolein (LnLnLn). Conditions: 0.45 m × 2.5 mm I.D. glass column packed with 2% Poly-S-179 on Supelcoport (100–120 mesh). Temperature programmed between 220–335° at 3°/min with a nitrogen flow-rate of 80 ml/min. Injection port and detector maintained at 350°. (B) Attempted resolution of tristearin (SSS), triolein (OOO) and trilinolein (LnLnLn) on OV-17 stationary phase. Conditions: 0.45 m × 2.5 mm I.D. glass column packed with 2% OV-17 on Supelcoport (100–120 mesh). Temperature programmed in the range 230–350° at 3° min with a nitrogen flow-rate of 80 ml/min. Injection port and detector maintained at 350°.

mum, as it gave good resolution and recovery of triglycerides without having to utilise temperatures beyond 350° to elute the higher molecular weight samples.

Poly-S-179 was the only stationary phase to resolve triglycerides of the same carbon number, but differing in degree of unsaturation, on a 0.45 m column. Fig. 2A shows a typical chromatogram for the resolution of a mixture of saturated and unsaturated triglycerides; the triglycerides are eluted in order of increasing molecular weight and increasing degree of unsaturation. Attempts to increase the resolution of the C_{54} triglycerides by moving to longer column lengths met with only partial success, as the increase in resolution was accompanied by a marked increase in the amount of on-column degradation. On a 1.06 m column containing 2% Poly-S-179, tristearin and triolein were completely resolved with baseline resolution but the chromatogram showed marked amounts of broad ill-defined material eluting immediately before the tristearin peak making a quantitative analysis troublesome. This on-column degradation places a practical limit on column length. Weight and molar response factors were calculated for triolein and trilinolein on the Poly-S-179 phase and these are given in Table III.

Fig. 2B shows the attempted resolution of tristearin, triolein and trilinolein on a 0.45 m column containing 2% OV-17. The saturated and unsaturated triglycerides were not resolved with trilinolein distorting the descending edge of the main C_{54} peak.

The reference mixture of simple saturated monoacid triglycerides was eluted from the 2% Poly-S-179 column at a temperature approximately 25° below that on the other stationary phases. This is probably due to the low affinity of the triglycerides, which have a non-polar character, for the polar stationary phase. The response factors for the unsaturated triglycerides increased with the degree of unsaturation, indicating an increase in irreversible adsorption onto the column and an increase in on-column degradation.

The present study is to be extended by utilising high-resolution capillary GLC columns coated with Poly-S-179. Lower elution temperatures and increased resolution should enable valuable separations of synthetic and natural triglyceride mixtures to be effected, based on both molecular weight and degree of unsaturation.

In conclusion, it can be said that a polyphenyl ether sulphone (Poly-S-179) stationary phase has sufficient polarity to permit improved separations of triglycerides based on the degree of unsaturation and sufficient thermal stability to allow its application without deterioration.

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