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SILAR 10 C, SILAR 9 CP, SP 2340 AND OV-275 IN THE GAS–LIQUID CHROMATOGRAPHY OF FATTY ACID METHYL ESTERS ON PACKED COLUMNS

CHROMATOGRAPHIC CHARACTERISTICS AND MOLECULAR STRUCTURES

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SUMMARY

Gas–liquid chromatographic characteristics of Silar 10 C, Silar 9 CP, SP 2340 and OV-275, which are recently developed silicone stationary phases of high polarity with good temperature stability, were studied on packed columns. The possibility of the identification and separation of positional and geometric isomers of mono-, di- and triethylenic fatty acid methyl esters is discussed. The chemical constitutions of the stationary phases investigated were determined.

INTRODUCTION

Unsaturated fatty acids from glycerides of vegetable origin occur almost exclusively in the *cis*-configuration, exceptions being plants of certain families[1,2]. *trans*-Unsaturated fatty acids cannot be synthesized as body-building materials by humans and animals, but such components, some of them in considerable amounts, have been found in all body tissues that have been investigated[1,3,4]. This suggests that these *trans*-fatty acids originate in the diet, either in margarines and cooking, baking and frying fats that have previously undergone industrial partial catalytic hydrogenation[5,6] or in foodstuffs derived from ruminants. The rumen or rumen-like

stomach of these animals has a microflora which, during the process of digestion, hydrogenates the unsaturated fatty acids contained in the fodder[7] and also partially isomerizes them[8].

The physiological nutritional importance of these *trans*-unsaturated fatty acids for the human organism, especially in cases of chronic ingestion[9,10], has not been elucidated so far despite numerous investigations, although the amounts ingested may be high, depending on the kind of food eaten.

Many methods have been described for the qualitative and quantitative analysis of *trans*-fatty acids in general, and of isooleic acid, the main product of catalytic and microbial partial hydrogenation, in particular. Reference is made here to the lead salt precipitation method of Twitchell[11] and its numerous modifications, to column chromatographic methods[12–14], thin-layer chromatographic techniques[15–18] (mostly on silica gel–silver nitrate layers) and to reversed-phase high-performance liquid chromatography[19]. The separation of geometric isomers by gas–liquid chromatography (GLC) has generally been carried out on capillary columns[12,20]. IR spectroscopy[21] is the commonly used method. All of these methods have drawbacks: some require a great deal of equipment and are time consuming, while others carry the risk of considerable errors, require greater amounts of substances or, as in IR spectroscopy, do not permit the differentiation of individual *trans*-fatty acids.

GLC on packed columns for the separation of *cis*/*trans*-isomers has hitherto been limited to substances that require maximum elution temperatures of 130° [22,23]. As temperature-resistant stationary phases of high polarity were not available, the separation of geometric isomers of fatty acids on packed columns was possible only after their conversion into epoxy fatty acid methyl esters on EGSS-X[24]. The development of the stationary phases Silar 10 C, Silar 9 CP, SP 2340 and OV-275, which are of high polarity and resistant to temperatures of at least 250°, has now made it possible to separate the geometric isomers of fatty acids on packed columns. We used these phases and thoroughly investigated their properties in connection with an extensive study of the composition of commercial fats intended for human consumption[6] and this paper summarizes our results.

EXPERIMENTAL

All pure samples of the numerous different fatty acid methyl esters tested were purchased from Nu-Check-Prep (Elysian, Minn., U.S.A.) and from Analabs (North Haven, Conn., U.S.A.). They were of known structure and more than 99% pure. Octadecadienoate and octadecatrienoate mixtures containing the mixed *cis*/*trans*-isomers were prepared from pure linoleic and linolenic standards according to the nitrous acid isomerization method of Litchfield and co-workers[25,26]. After permethylation[27], further purification was carried out on preparative silica gel H plates impregnated with silver ions[15]. Pure standards of 9-*trans*-12-*cis*- and 9-*cis*-12-*trans*-octadecadienoic fatty acids were a generous gift from Th. Wieske, Union Deutsche Lebensmittelwerke (Hamburg, G.F.R.).

The compounds for measurement of the McReynolds constants were commercial products from Applied Science Labs. (State College, Pa., U.S.A.). The stationary phases Silar 10 C and Silar 9 CP were also obtained from Applied Science Labs., SP 2340 and OV-275 were products from Supelco (Bellefonte, Pa., U.S.A.)

and the support Chromosorb W HP, 80–100 mesh, was purchased from Johns-Manville (Denver, Colo., U.S.A.).

Gas–liquid chromatographic analysis

A Hewlett-Packard 5830A chromatograph equipped with a flame-ionization detector (FID) was used for most of the GLC analyses. Occasionally, a Perkin-Elmer F 20 B gas chromatograph also equipped with an FID and an integrator (Model 3373B, Hewlett-Packard) was used, as mentioned in Table I. The operating conditions for the analyses are described in the tables. Coiled glass columns (3.6 m × 0.2 cm I.D. and sometimes 4 m × 0.2 cm I.D.) were employed. The columns were filled with packing material (home-made) under vacuum and with gentle vibration.

RESULTS AND DISCUSSION

The separation of seven pairs of *cis/trans*-isomeric unsaturated fatty acid methyl esters on a conventionally packed column with 12% Silar 10 C as the stationary phase is shown in Fig. 1. Baseline separation of the all-*cis* from the all-*trans* components of octadecadiethylenic and octadecatriethylenic fatty acids was achieved. The geometric pairs of monoenes, which were completely separated from each other, showed different degrees of peak overlap for *cis/trans*-components with a minimum for pentadecenoic acids and a maximum for octadecenoic acids.

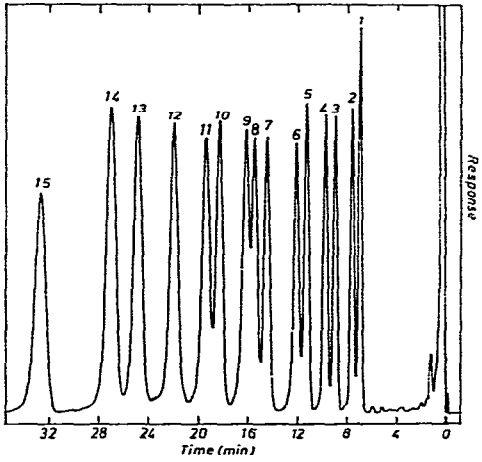


Fig. 1. Chromatogram of mixture of seven pairs of *cis/trans*-isomeric unsaturated fatty acid methyl esters. 1 = $C_{14:1}\Delta 9t$ (t = *trans*); 2 = $C_{14:1}\Delta 9c$ (c = *cis*); 3 = $C_{15:1}\Delta 10t$; 4 = $C_{15:1}\Delta 10c$; 5 = $C_{16:1}\Delta 9t$; 6 = $C_{16:1}\Delta 9c$; 7 = $C_{17:1}\Delta 10t$; 8 = $C_{17:1}\Delta 10c$; 9 = $C_{18:0}$; 10 = $C_{18:1}\Delta 9t$; 11 = $C_{18:1}\Delta 9c$; 12 = $C_{18:2}\Delta 9t,12t$; 13 = $C_{18:2}\Delta 9c,12c$; 14 = $C_{18:3}\Delta 9t,12t,15t$; 15 = $C_{18:3}\Delta 9c,12c,15c$. For operating conditions, see Table I.

Erucic and brassidic acids were also separated satisfactorily, but with a lower resolution than oleic/elaidic acids, as shown in Fig. 2. Mixed 9-*cis*-12-*trans*- and 9-*trans*-12-*cis*-octadecenoic acids were eluted together from the column, giving a single peak insignificantly broadened compared with mono-component peaks. This peak

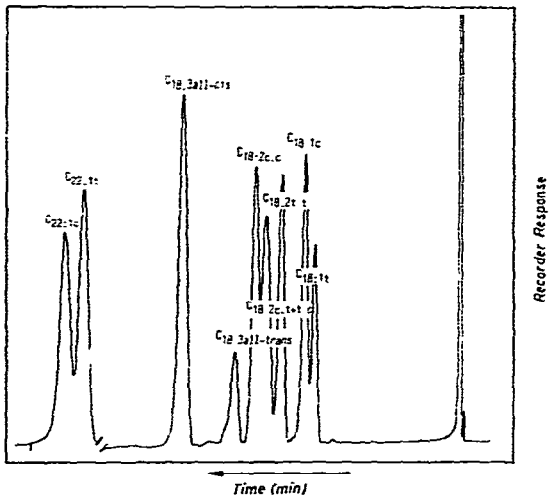


Fig. 2. Fractogram of methyl esters of isomeric octadecadiethylenic and of brassidic/erucic acids completed by pairs octadecamonoethylenic and octadecatriethylenic acids. Coiled glass column, 4 m × 0.2 cm I.D., packed with 12% Silar 10 C (charge A) on Chromosorb W HP, 80–100 mesh. For operating conditions, see Table I.

was not completely separated from that of linolelaidic acid and showed considerable overlap with that of linoleic acid, as demonstrated in Fig. 2.

Even the eight possible geometric isomers of methyl linolenate, obtained after isomerization of linolenic acid with nitrous acid[28], could be partially resolved on the 12% Silar 10 C packed column. As shown in Fig. 3, the six mixed geometric isomers

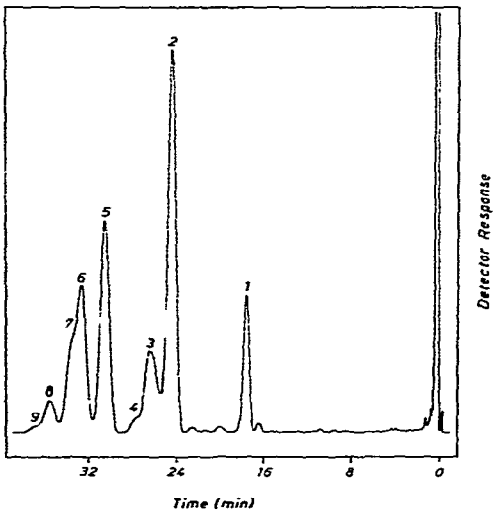


Fig. 3. Chromatogram of fatty acid methyl esters derived from isomerized linoleic and linolenic acid on 12% Silar 10 C. 1 = $C_{18:0}$; 2 = $C_{18:2}\Delta 9t,12t$; 3 = $C_{18:2}\Delta 9t,12c$ and $C_{18:2}\Delta 9c,12t$ (*in equal amounts*); 4 = $C_{18:2}\Delta 9c,12c$; 5 = $C_{18:3}\Delta 9t,12t,15t$; 6 and 7 = all $C_{18:3}\Delta$di-*trans*-mono-*cis* isomers (tentatively identified); 8 = all $C_{18:3}\Delta$di-*cis*-mono-*trans* isomers (tentatively identified). For operating conditions, see Table I.

resulted in two broadened peaks, one of which contained a shoulder, with nearly complete resolution or with at least an adequate resolution from each other and from the all-*trans* or all-*cis* components to allow their quantification. As we carried out no further identification experiments, the elution pattern of these mixed triethylenic isomers could not be identified with absolute certainty. Based on (a) the retention data for Silar 10 C, which are subsequently discussed in detail, (b) the known composition of the isomerized mixture[28] and (c) the appearance of the chromatogram (Fig. 3), it

TABLE I

RELATIVE RETENTION TIMES (RRT) AND EQUIVALENT CHAIN LENGTHS (ECL) OF FATTY ACID METHYL ESTERS ON SILAR 10 C AS STATIONARY PHASE

Analyses were performed on a Perkin-Elmer gas chromatograph, Model 20B, equipped with an FID. The chromatograph was fitted with a glass column (4 m × 0.2 cm I.D.), packed with Chromosorb W HP, 80–100 mesh and coated with 12% Silar 10 C (charge A). The column was operated at 170° with nitrogen at a flow-rate of 10 ml/min) 1.3 atm (as carrier gas. Injector and detector temperatures, 250°; sample size, 0.1–0.2 μl in chloroform (3.5%, w/v).

Fatty acid	*ECL*	*RRT**	*Fatty acid*	*ECL*	*RRT**
6:0		0.05	19:0		1.28
8:0		0.08	18:2Δ 9t, 12t	19.31	1.38
10:0		0.14	*iso*-20:0	19.43	1.42
11:0		0.17	19:1Δ 7t	19.49	1.44
12:0		0.227	19:1Δ10t	19.54	1.46
anteiso-13:0	12.56	0.26	19:1Δ12t	19.56	1.47
13:0		0.29	18:2Δ 9c, 12t	19.58	1.48
12:1Δ11c	13.12	0.30	18:2Δ 9t, 12c	19.65	1.50
iso-14:0	13.43	0.32	19:1Δ 7c	19.70	1.52
14:0		0.37	18:2Δ 9c, 12c	19.71	1.53
anteiso-15:0	14.56	0.44	19:1Δ10c	19.78	1.55
14:1Δ 9t	14.75	0.45	19:1Δ12c	19.85	1.58
15:0		0.476	20:0		1.64
14:1Δ 9c	15.04	0.48	18:3Δ 9t, 12t, 15t	20.17	1.71
iso-16:0	15.43	0.53	18:3Δ 6c, 9c, 12c	20.51	1.86
15:1Δ10t	15.78	0.57	20:1Δ 8c	20.70	1.95
15:1Δ10c	16.04	0.61	20:1Δ11c	20.77	1.98
16:0		0.61	20:1Δ 13c	20.84	2.02
20:0**	16.44	0.68	18:3Δ 9c, 12c, 15c	20.91	2.08
anteiso-17:0	16.56	0.70	21:0		2.10
16:1Δ9t	16.61	0.71	20:2Δ11c, 14c	21.71	2.51
16:1Δ9c	16.89	0.76	22:0		2.69
17:0		0.78	20:3Δ8c, 11c, 14c	22.51	3.04
iso-18:0	17.43	0.86	22:1Δ13t	22.54	3.07
17:1Δ10t	17.61	0.91	22:1Δ13c	22.77	3.25
17:1Δ10c	17.89	0.97	20:4Δ5c, 8c, 11c, 14c	22.92	3.37
18:0		1.00	23:0		3.44
18:1Δ6t	18.49	1.13	22:2Δ13c, 16c	23.71	4.11
18:1Δ9t	18.54	1.14	24:0		4.40
18:1Δ11t	18.56	1.15	20:5Δ5c, 8c, 11c, 14c, 17c	24.08	4.48
anteiso-19:0	18.56	1.15	22:3Δ13c, 16c, 19c	24.91	5.49
18:1Δ6c	18.71	1.19	22:4Δ7c, 10c, 13c, 16c	24.92	5.515
18:1Δ9c	18.77	1.21	26:0		7.22
18:1Δ11c	18.84	1.23	22:6Δ4c, 7c, 10c, 13c, 16c, 19c	27.16	8.00

* Relative retention time for stearic acid methyl ester (retention time 16.5 min) taken as 1.00.

** Phytanic acid methyl ester (3,7,11,15-tetramethylhexadecanoic acid methyl ester).

can be concluded that peak 6, including the shoulder which is marked as peak 7, contains predominantly di-*trans*-isomers, whereas peak 8 is composed of di-*cis*-isomers.

As retention data can be conveniently presented in the form of equivalent chain length (ECL) values ,we studied the ECL values and the relative retention times for a number of saturated, geometrical and positional monoethylenic and polyethylenic fatty acids on 12% Silar 10 C. Retention times were measured directly from the chromatograms as retention distances. At least three determinations were made in each instance. Retention times on a 4 m × 0.2 cm coiled packed glass column coated with 12% Silar 10 C under optimized conditions are summarized in Table I. The logarithms of the recorded relative retention times of fatty acid methyl esters are plotted against the number of carbon atoms in these standards in Figs. 4 and 5.

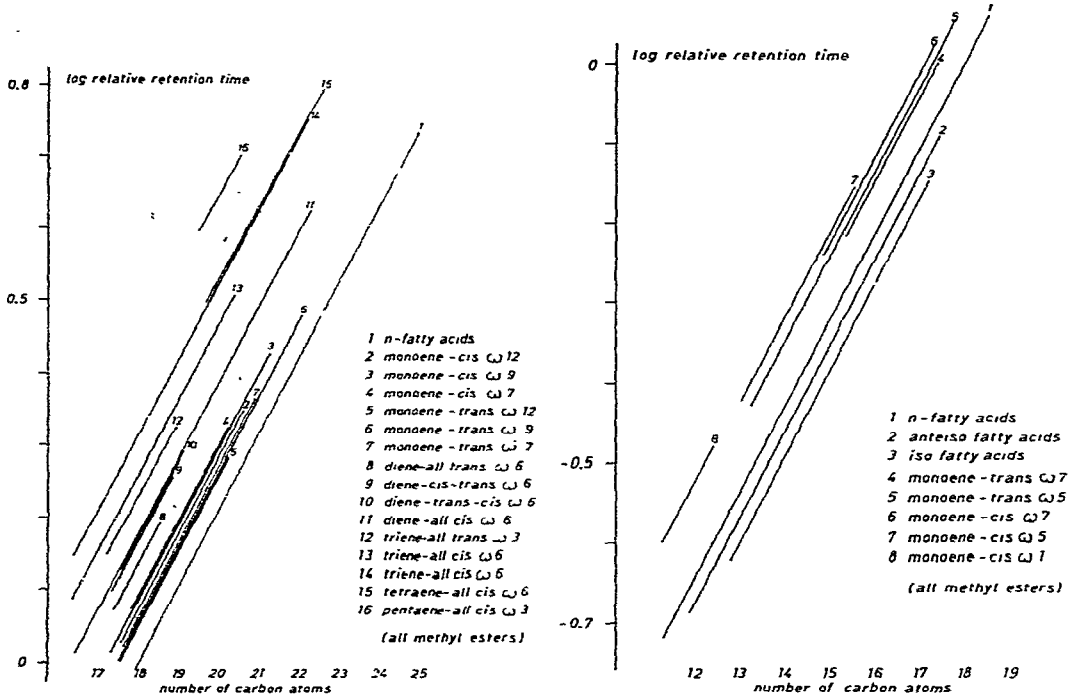


Fig. 4. Correlation between logarithm of relative retention time (stearic acid methyl ester = 1.00) and number of carbon atoms for different fatty acid methyl esters on 12% Silar 10 C (Charge A) at 170°. For conditions, see Table I.

Fig. 5. As Fig. 4 for further compounds.

The *anteiso*-branching and even more the *iso*-branching in the alkyl chain reduced the retention time. Each double bond in the alkyl chain caused a distinct increase in retention time; for *cis*-ethylenic bonds the increments were greater than for the corresponding *trans*-ethylenic bonds. The retention times of positional isomers of equivalent geometric monoethylenic acids increased continuously when the double bond was removed from the carbonyl group; this behaviour on polyester columns has long been recognized[29]. In comparison with *trans*-monoethylenic bonds these incre-

ments became greater for *cis*-bonds. Analogous to these data, *cis,trans*-diethylenic acids gave smaller increments in retention time than the isomeric *trans,cis*-diethylenic acids. Although we found small but constant differences in retention times for individual *cis*- and *trans*-positional isomers, it was impossible for practical purposes to separate positional isomers from component mixtures. A comparison of the retention data in Table I further indicates that some commonly found acids show considerable overlap with others in complex mixtures. However, GLC analysis on Silar 10 C was useful when the complex sample was additionally analyzed on an EGA packed column.

TABLE II

McREYNOLDS CONSTANTS FOR SILAR 10 C (CHARGE D) AND SP 2340

Retention indices given are mean values from three different prepared packings. Each packing had been tested at least five times. The deviation of the mean values from different packings was found to be within the range ± 3.

Compound	*Silar 10 C*	*SP 2340*		*Silar 10 C*	*SP 2340*
X′ = *ΔI* Benzene	526	522	H = *ΔI* 2-Methyl-2-pentanol	602	606
Y′ = *ΔI* Butanol	760	763	J = *ΔI* 1-Iodobutane	481	478
Z′ = *ΔI* 2-Pentanone	665	661	K = *ΔI* 2-Octyne	306	301
U′ = *ΔI* 1-Nitropropane	954	948	L = *ΔI* 1,4-Dioxane	731	733
S′ = *ΔI* Pyridine	812	807	M = *ΔI cis*-Hydrindane	267	259

As the McReynolds constants for Silar 10 C and SP 2340 are said to be identical[30] and as our results of retention index measurements, compiled in Table II, showed small differences from each other and from Supina's values[30], it was obvious that we should conduct a resolution study on both stationary phases. The results of the comparative measurements of resolution on Silar 10 C and SP 2340, completed by Silar 9 CP and OV-275 under varying conditions, are summarized in Tables III and IV. The resolution for *cis*/*trans*-isomeric pairs of monounsaturated fatty acids decreased both with increasing oven temperature and increasing nitrogen flow-rate on all four stationary phases. The best resolutions for Silar 10 C, Silar 9 CP and SP 2340 were obtained at 170° and for OV-275 at 150 °C, with a nitrogen flow-rate of 10 ml/min. The best resolutions were obtained with 10% and 12% stationary phase concentrations. Taking into account also the retention times for the five isomers under consideration, a 10% stationary phase concentration proved to be more advantageous than 12% for Silar 10 C and SP 2340. As we initially believed that 12% packings of Silar 10 C and SP 2340 would be more effective for the separation of the geometric isomeric fatty acid mixture studied, we conducted a comparative study with 12% Silar 9 CP and 12% OV-275. All of the packings were found to have considerable molecular similarities and to be suitable for separating the geometric isomers of fatty acid methyl esters. Fig. 6 shows the differences in the GLC characteristics for these four phases of high polarity. Analyses on higher (15%) and lower (3% and 7%) concentration packings of Silar 10 C and SP 2340 resulted in decreased resolution. Because of the poorer performance, especially for the lesser coated supports, further resolutions are not tabulated here; they are illustrated indirectly by the separation factors summarized in Table V.

ABLE III

ESOLUTION OF DIFFERENT *CIS/TRANS*-MONOETHYLENIC FATTY ACID METHYL ESTERS O ACKED GLASS COLUMNS AT DIFFERENT NITROGEN FLOW-RATES AND OVEN TEMPERATURE

olumns: 3.6 m × 0.2 cm I.D., coated with (1) 12% Silar 10 C, (2) 12% Silar 9 CP, (3) 12% SP 2340 and (4) 12 V-275 on Chromosorb W HP, 80–100 mesh. Chromatograph: Hewlett-Packard 5830A. Resolution was calculate ; $R = 2\,(t_{R_2} - t_{R1})/(W_1 + W_2)$. Values for 12% OV-275 were measured exceptionally at 150°, 160° and 170° wi itrogen flow-rates of 10, 15 and 20 ml/min. n.s. means no separation. The number of theoretical plates for myrist idic acid methyl ester was calculated from the equation $N = (t_{m+s}/b_{0.5})^2 \cdot 5.54132$. t_R = retention time, W = eak width, t_{m+s} = uncorrected retention time, $b_{0.5}$ = peak width at half height.

sters	Column	*10 ml/min* N_2				*15 ml/min* N_2				*20 ml/min* N_2			
		170°	*175°*	*180°*	*185°*	*170°*	*175°*	*180°*	*185°*	*170°*	*175°*	*180°*	*185°*
$C_{14:1t}/C_{14:1c}$	1	1.07	0.98	0.94	0.91	0.98	0.92	0.86	0.79	0.91	0.81	0.81	0.74
Δ9	2	1.13	1.05	1.03	0.92	1.05	0.99	0.91	0.89	0.97	0.93	0.86	0.78
	3	1.15	1.08	1.01	0.98	1.02	1.00	0.89	0.85	0.99	0.94	0.90	0.77
	4	1.13	1.05	0.92		1.05	0.91	0.77		0.97	0.88	0.80	
$C_{14:1c}/C_{15:1t}$	1	2.67	2.33	2.15	1.98	2.34	2.22	1.95	1.68	2.17	2.01	1.80	1.63
	2	3.69	3.33	3.07	2.68	3.35	3.08	2.68	2.47	3.04	2.87	2.54	2.32
	3	2.71	2.42	2.33	2.04	2.53	2.35	2.00	1.86	2.46	2.12	2.05	1.75
	4	2.00	1.59	1.29		1.83	1.42	1.12		1.68	1.35	1.09	
$C_{15:1t}/C_{15:1c}$	1	1.12	1.05	1.03	1.02	1.05	1.02	0.91	0.89	0.96	0.92	0.89	0.81
Δ10	2	1.15	1.13	1.06	1.04	1.08	1.05	0.96	0.93	1.00	0.97	0.92	0.90
	3	1.17	1.08	1.07	0.98	1.07	1.00	0.97	0.92	0.99	0.99	0.93	0.90
	4	1.19	1.11	1.00		1.09	0.95	0.88		0.98	0.91	0.84	
$_{15:1c}/C_{16:1t}$	1	2.47	2.26	2.05	1.88	2.23	2.08	1.87	1.67	2.03	1.87	1.74	1.53
	2	3.46	3.28	2.92	2.75	3.22	2.96	2.63	2.39	2.97	2.73	2.51	2.25
	3	2.52	2.25	2.13	1.97	2.35	2.19	1.98	1.80	2.22	2.00	1.86	1.69
	4	1.84	1.48	1.17		1.71	1.30	1.01		1.53	1.24	0.99	
$C_{16:1t}/C_{16:1c}$	1	0.99	0.95	0.89	0.83	0.90	0.84	0.81	0.76	0.77	0.76	0.71	0.64
Δ9	2	0.92	0.89	0.89	0.83	0.95	0.91	0.86	0.82	0.86	0.80	0.78	0.72
	3	1.00	0.97	0.95	0.89	0.93	0.92	0.89	0.86	0.88	0.85	0.80	0.76
	4	1.19	0.97	0.80		0.96	0.84	0.77		0.88	0.78	0.74	
$_{16:1c}/C_{17:1t}$	1	3.17	3.00	2.73	2.41	2.92	2.67	2.40	2.14	2.67	2.42	2.17	1.90
	2	4.43	4.01	3.68	3.48	4.03	3.77	3.42	3.09	3.80	3.46	3.05	2.77
	3	3.39	3.11	2.85	2.59	3.19	2.88	2.56	2.41	2.94	2.67	2.45	2.25
	4	2.42	2.14	1.73		2.39	1.88	1.47		2.13	1.72	1.42	
$C_{17:1t}/C_{17:1c}$	1	1.01	0.91	0.87	0.82	0.84	0.88	0.78	0.70	0.82	0.76	0.72	0.68
Δ10	2	0.98	0.98	0.95	0.90	0.92	0.89	0.89	0.89	0.84	0.81	0.82	0.75
	3	0.97	0.96	0.99	0.89	0.91	0.91	0.87	0.77	0.90	0.91	0.86	0.78
	4	1.14	—	—	—	0.90	—	—	—	—	—	—	—
$_{17:1c}/C_{18:0}$	1	1.05	0.93	0.64	0.40	1.01	0.76	0.59	0.38	0.79	0.68	0.51	0.33
	2	1.99	1.84	1.56	1.45	1.95	1.72	1.47	1.26	1.79	1.58	1.39	1.16
	3	1.14	0.97	0.71	0.50	1 05	0.84	0.65	0.46	0.97	0.77	0.53	0.36
	4	0.50	n.s.	n.s.	n.s.	0.28	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$_{18:0}/C_{18:1t}$	1	2.10	2.00	1.84	1.59	1.88	1.81	1.54	1.44	1.71	1.58	1.37	1.31
	2	2.21	2.13	2.05	1.95	2.06	1.93	1.83	1.81	1.91	1.83	1.76	1.68
	3	2.06	2.06	2.00	1.85	1.89	1.91	1.66	1.53	1.81	1.79	1.58	1.50
	4	1.92	1.80	1.65		1.73	1.60	1.59		1.37	1.51	1.36	
$_{17:1c}/C_{18:1t}$	1	3.12	2.96	2.56	2.09	2.84	2.57	2.21	1.77	2.56	2.30	2.03	1.72
	2	4.10	3.91	3.51	3.37	3.84	3.44	3.23	3.01	3.56	3.32	3.02	2.76
	3	3.24	3.05	2.80	2.37	2.94	2.82	2.44	2.22	2.76	2.58	2.13	1.77
	4	2.58	—	—	—	2.01	—	—	—	—	—	—	—
$_{18:1t}/C_{18:1c}$	1	0.86	0.86	0.83	0.77	0.75	0.75	0.75	0.65	0.62	0.66	0.59	0.56
Δ9	2	0.80	0.80	0.77	0.76	0.72	0.71	0.69	0.67	0.67	0.64	0.61	0.59
	3	0.81	0.83	0.88	0.83	0.73	0.78	0.76	0.76	0.74	0.69	0.70	0.63
	4	0.99	0.91	0.85		0.89	0.80	0.73		0.74	0.72	0.69	

TABLE III *(continued)*

Esters	*Column*	*10 ml/min N_2*				*15 ml/min N_2*				*20 ml/min N_2*			
		170°	*175°*	*180°*	*185°*	*170°*	*175°*	*180°*	*185°*	*170°*	*175°*	*180°*	*185°*
$NC_{14:1t/m}$	1	855	726	694	676	660	605	602	532	574	509	494	451
	2	1083	1038	998	863	995	851	726	697	762	744	674	608
	3	929	872	841	795	803	812	720	600	706	620	595	485
	4	595	551	484		500	417	144		403	386	90	
Retention time	1	41.73	36.05	29.25	23.87	33.00	27.00	22.00	17.95	27.96	22.73	18.49	15.05
(min),	2	58.03	47.00	38.19	31.19	44.90	36.14	29.29	23.86	37.82	30.31	24.53	19.95
$C_{18:1c}$	3	36.19	30.43	26.83	21.51	28.95	24.47	21.63	17.89	27.77	22.89	19.01	15.23
(gross)	4	45.03	27.61	18.15		33.20	20.78	13.65		27.84	17.75	11.53	

TABLE IV

RESOLUTION OF DIFFERENT *CIS/TRANS*-MONOETHYLENIC FATTY ACID METHYL ESTERS ON PACKED GLASS COLUMNS AT DIFFERENT NITROGEN FLOW-RATES AND OVEN TEMPERATURES

Columns: 3.6 m × 0.2 cm I.D., coated with (1) 10% Silar 10 C and (2) 10% SP 2340 on Chromosorb W HP, 80–100 mesh. Chromatograph: Hewlett-Packard 5830A. Details as in Table III.

Esters	*Column*	*10 ml/min N_2*				*15 ml/min N_2*				*20 ml/min N_2*			
		170°	*175°*	*180°*	*185°*	*170°*	*175°*	*180°*	*185°*	*170°*	*175°*	*180°*	*185°*
$\frac{C_{14:1t}/C_{14:1c}}{\Delta 9}$	1	1.11	1.04	0.96	0.93	0.99	0.92	0.91	0.71	0.86	0.78	0.73	0.71
	2	1.08	1.02	0.93	0.95	1.00	0.92	0.85	0.80	0.93	0.83	0.82	0.80
$C_{14:1c}/C_{15:1t}$	1	2.69	2.43	2.23	2.01	2.48	2.22	1.99	1.59	2.24	1.93	1.61	1.57
	2	2.87	2.46	2.27	2.15	2.53	2.31	2.12	1.77	2.40	2.04	1.89	1.60
$\frac{C_{15:1t}/C_{15:1c}}{\Delta 10}$	1	1.13	1.09	1.05	1.01	0.98	0.94	1.00	0.83	0.92	0.90	0.80	0.81
	2	1.16	1.14	1.07	1.05	1.02	1.04	0.95	0.89	1.03	0.95	0.92	0.85
$C_{15:1c}/C_{16:1t}$	1	2.51	2.38	2.11	1.92	2.38	2.12	1.97	1.56	2.08	1.86	1.62	1.52
	2	2.80	2.44	2.23	2.06	2.46	2.17	2.03	1.77	2.28	2.05	1.76	1.68
$\frac{C_{16:1t}/C_{16:1c}}{\Delta 9}$	1	0.94	0.91	0.86	0.85	0.88	0.83	0.84	0.64	0.83	0.76	0.70	0.73
	2	0.98	0.92	0.88	0.90	0.87	0.88	0.85	0.79	0.86	0.75	0.76	0.76
$C_{16:1c}/C_{17:1t}$	1	3.26	3.08	2.71	2.49	3.04	2.78	2.51	1.96	2.71	2.41	2.12	2.02
	2	3.58	3.21	2.94	2.77	3.12	2.95	2.65	2.37	2.99	2.68	2.45	2.22
$\frac{C_{17:1t}/C_{17:1c}}{\Delta 10}$	1	1.03	1.01	0.93	0.88	0.88	0.90	0.82	0.66	0.81	0.81	0.69	0.65
	2	0.99	0.91	0.91	0.91	0.90	0.89	0.87	0.80	0.88	0.80	0.80	0.76
$C_{17:1c}/C_{18:0}$	1	1.12	0.88	0.73	0.51	1.02	0.89	0.62	0.32	0.95	0.70	0.53	0.34
	2	1.33	1.15	0.93	0.76	1.28	1.09	0.82	0.55	1.10	0.92	0.74	0.59
$C_{18:0}/C_{18:1t}$	1	2.14	2.09	1.97	1.60	1.95	1.81	1.67	1.19	1.74	1.65	1.34	1.07
	2	2.10	2.04	2.02	1.93	1.94	1.87	1.80	1.56	1.85	1.72	1.65	1.61
$C_{17:1c}/C_{18:1t}$	1	3.03	2.94	2.73	2.33	2.98	2.72	2.35	1.82	2.68	2.37	1.98	1.42
	2	3.40	3.11	2.98	2.75	3.16	2.98	2.60	2.19	2.89	2.55	2.37	2.19
$\frac{C_{18:1t}/C_{18:1c}}{\Delta 9}$	1	0.89	0.89	0.78	0.81	0.79	0.74	0.72	0.61	0.76	0.70	0.61	0.46
	2	0.87	0.80	0.86	0.79	0.70	0.71	0.71	0.71	0.66	0.70	0.68	0.68
$NC_{14:1t/m}$	1	904	857	807	803	764	716	633	492	631	523	431	468
	2	960	890	812	757	842	765	655	571	735	562	556	577
Retention time	1	33.62	27.29	22.23	18.31	25.81	20.83	17.06	14.20	21.57	17.60	14.46	11.84
(min)	2	33.23	26.63	22.46	18.53	25.73	21.23	17.44	14.66	21.57	18.79	15.26	12.33
$C_{18:1c}$ (gross)													

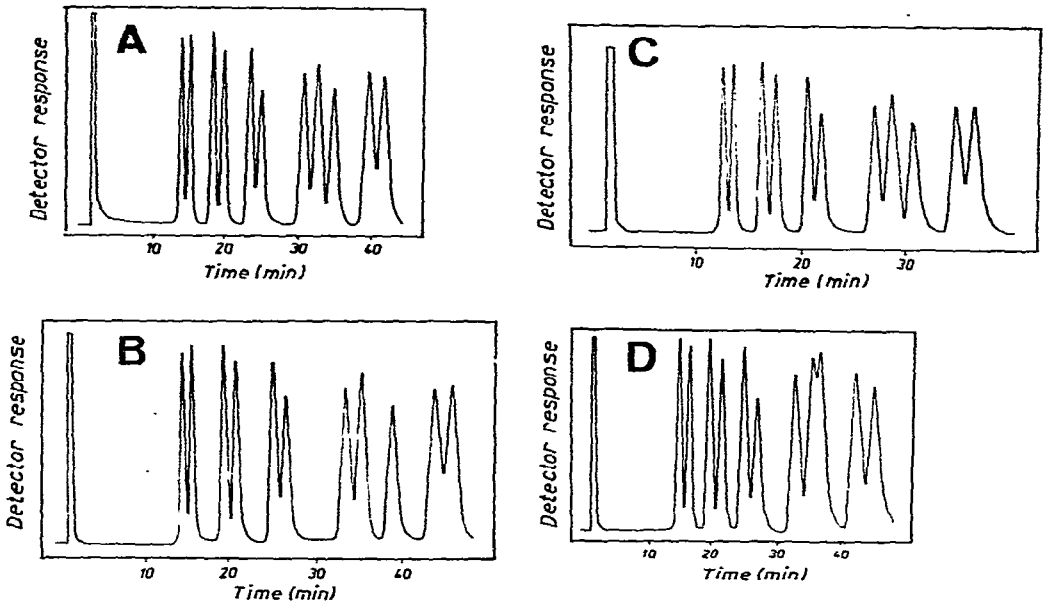


Fig. 6. Chromatograms of pairs of *cis/trans*-monoethylenic fatty acid methyl esters on (A) 12% Silar 10 C (charge D), (B) 12% Silar 9 CP, (C) 12% SP 2340 and (D) 12% OV-275. Fatty acid methyl ester mixture, corresponding to the elution order, is composed of: $C_{14:1}\Delta 9t$; $C_{14:1}\Delta 9c$; $C_{15:1}\Delta 10t$; $C_{15:1}\Delta 10c$; $C_{16:1}\Delta 9t$; $C_{16:1}\Delta 9c$; $C_{17:1}\Delta 10t$; $C_{17:1}\Delta 10c$; $C_{18:0}$; $C_{18:1}\Delta 9t$; $C_{18:1}\Delta 9c$. Chromatograph: Hewlett-Packard, Model 5830A, fitted with double FID. Column: glass, 3.6 m × 0.2 cm I.D. Support: Chromosorb W HP, 80–100 mesh. Carrier gas: nitrogen at 10 ml/min. Column temperatures: for A, B and C, 170°; for D, 150°. Injection port temperature: 250°.

Although the McReynolds constants obtained with Silar 10 C were very similar to those with SP 2340, a choice between these two stationary phases had to be made for practical purposes on the basis of other properties. Both stationary phases had high thermal stability (250°), with no noticeable bleeding when operated at 170–185° and with no loss of performance when used for more than 500 analyses. Higher temperature ranges for a longer time were not tested because temperature-programmed analyses of samples containing a multiplicity of fatty acids (Table I) revealed lower resolutions. While the chemical properties and the molecular structures of different charges of SP 2340, Silar 9 CP and OV-275, purchased repeatedly over a 2-year period, remained constant, different charges of Silar 10 C exhibited considerable differences in chemical composition and did not have identical properties for our chromatographic purposes. Samples of Silar 10 C with high viscosity that were semi-solid, crumbly and opaque at room temperature, soluble only in acetonitrile and insoluble in chloroform and other solvents were unsuitable as they gave poor resolution. Silar 10 C suitable for the separation of geometric isomers of fatty acid methyl esters is clear and colourless, has a honey-like viscosity and dissolves easily in chloroform. Consequently, Silar 10 C cannot be recommended without reservations in comparison with SP 2340.

For improved characterization of stationary phases A–F, ^{1}H NMR and IR spectra were measured .The ^{1}H NMR spectra are shown in Fig. 7. Apart from signal

TABLE V

SEPARATION FACTORS FOR PAIRS OF *cis*- AND *trans*-ISOMERS OF MONOUNSATURATED FATTY ACID METHYL ESTERS BY GLC ON PACKED COLUMNS

Columns: glass, 3.6 m × 0.2 cm I.D., coated with (a) Silar 10 C (charge D), (b) SP 2340, (c) Silar 9 CP and (d) OV-275 at 170°, 175°, 180° and 185° and with nitrogen flow-rates of 10, 15 and 20 ml/min. Exceptionally, temperatures tested for OV-275 were 150°, 160° and 170°. Support: Chromosorb W HP, 80–100 mesh. Chromatograph: Hewlett-Packard, Model 5830A, fitted with an FID. Injection and detector temperatures: 250°. For separation factor measurement, measured retention times were corrected for dead volume by deduction of the retention time for methane.

Isomers	*Column*	*Stationary phase concentration (%)*				
		3	*7*	*10*	*12*	*15*
cis/trans-14:1Δ9	a	1.08	1.08	1.09	1.09	1.09
	b	1.08	1.08	1.09	1.09	1.09
	c				1.08	
	d				1.10	
cis/trans-15:1Δ10	a	1.07	1.08	1.09	1.09	1.09
	b	1.07	1.08	1.09	1.09	1.09
	c				1.08	
	d				1.10	
cis/trans-16:1Δ9	a	1.06	1.07	1.07	1.07	1.07
	b	1.06	1.07	1.07	1.07	1.07
	c				1.06	
	d				1.09	
cis/trans-17:1Δ10	a	1.05	1.06	1.07	1.07	1.07
	b	1 05	1.06	1.07	1.07	1.07
	c				1.06	
	d				1.09	
cis/trans-18:1Δ9	a	1.04	1.05	1.06	1.06	1.06
	b	1.03	1.05	1.06	1.06	1.06
	c				1.05	
	d				1.08	

4, which results from the solvent used (CD_3CN), and the tetramethylsilane signal 1, four signal groupings can be seen in each spectrum .The following assignments can be made for the spectra A and B: the triplet at 2.42 ppm (signal 7), the multiplet at 1.70 ppm (signal 3) and the multiplet at 0.78 ppm (signal 2) with an intensity ratio of 1:1:1 can be ascribed to the protons of $NC–CH_2–CH_2–CH_2–Si$ group. We propose that the signal at 0.16 ppm (signal 10) corresponds to a $(CH_3)_3Si$ group*.

The molecular structures are given in Table VI.

The essential difference between spectra A and B lies only in the ratios of the intensity of signal 10 to those of signals 2 and 7. Provided that all end-groups R^1 of the structure unit are trimethylsilyl groups [$R^1 = Si(CH_3)_3$], an averaged molecular weight can be calculated from the intensity ratio. For spectrum A this averaged n is about 22 and therefore the averaged molecular weight is about 4200; for B, n is calculated to be 56 (molecular weight 10,000). The difference in molecular weight between A and B is also apparent from a visual comparison and from the solubility

* An alternative but unlikely interpretation would be a $(CH_3)_2R–Si$ structure unit, so that the phases A, B and C would be a mixed methylcyanopropylsiloxane (R = H) with methyl to cyanopropyl ratios of about 1:7 (A), 1:18 (B) and 1:19 (C).

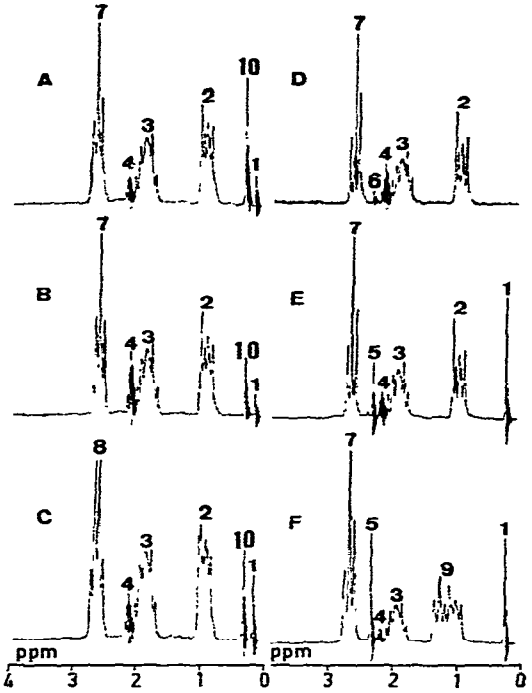
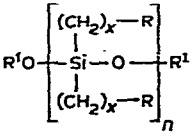


Fig. 7. ^1H NMR spectra of the stationary phases Silar 10 C (A–D), SP 2340 (E) and OV-275 (F), recorded with a JEOL JNM MH-100 at room temperature in CD_3CN. All chemical shifts are given in parts per million from tetramethylsilane.

characteristics of the two samples, as mentioned above. Spectrum C differs from A and B with respect to signal 8 at 2.4 ppm. It appears not as a single triplet, but as two overlapping triplets. One can therefore interpret this result by suggesting that in addition to the CH_2–CN group, the presence of the CN group being proved by the IR

TABLE VI
MOLECULAR STRUCTURES OF STATIONARY PHASES



Stationary phase	*R*	*x*	*R*^{1}	*n*
A	CN	3	$Si(CH_3)_3$	22
B	CN	3	$Si(CH_3)_3$	56
C	CN and partially Br (?)	3	$Si(CH_3)_3$	18
D	CN	3	H (?)	?
E	CN	3	$COCH_3$	15
F	CN	2.5	$COCH_3$	12
G	CN	3	$Si(CH_3)_3$	18

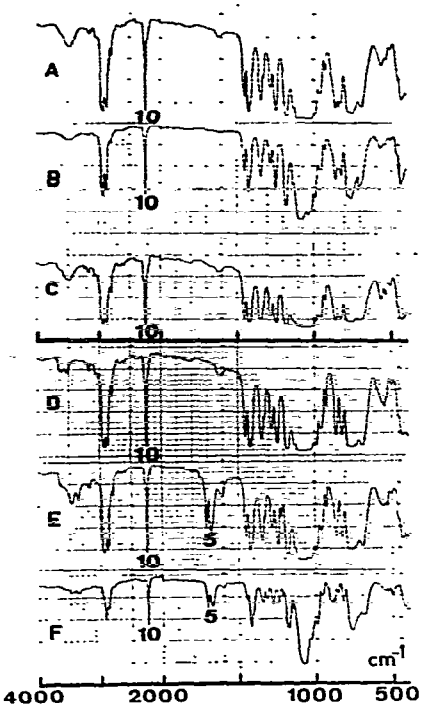


Fig. 8. IR spectra of the stationary phases Silar 10 C (A–D), SP 2340 (E) and OV-275 (F), recorded with a Perkin-Elmer IR 225 spectrometer (KI pellet).

spectra (Fig. 8), there is another CH_2–X grouping in which X possesses electronic properties similar to those of CN, as indicated by the similar chemical shift. The substituent could be a Br atom, for example. For spectrum C, the averaged n is 18 (molecular weight 3400). In spectrum D the signal of the trimethylsilyl group (signal 10) is completely absent, while signals 2, 3 and 7 are identical with those in spectra A and B. In addition, another, although weak, signal appears at 2.08 ppm, which cannot be clearly assigned. Therefore, stationary phase D differs from A, B and C in that no $(CH_3)_3Si$ groups are present as end-groups. In general, it can be said that stationary phases A–D, all labelled as Silar 10 C (recently Apolar) by the manufacturers, have different compositions. While it is true that A and B possess the same structural element, their molecular weights differ by a factor of more than 2.

The spectra of stationary phases E (SP 2340) and F (OV-275) likewise show no $(CH_3)_3Si$ groupings. However, a singlet appears at 2.07 ppm (signal 5). Together with the fact that the IR spectrum shows a carbonyl vibration at 1700 cm^{-1} in addition to the CN vibration at 2250 cm^{-1}, it can be concluded that the end-groups R^1 in stationary phases E and F are acetyl groups. The singlet at 2.07 ppm is then attributed to the CH_3 group of the acetate substituent. Provided that this is the case, n is calculated to be 15 for E (averaged molecular weight 2800). For F, the relationships are complicated because the intensity ratio of signals 7, 3 and 9 is no longer 1:1:1. Signal 3 shows a lower intensity, an explanation for which could be that an ethylene fragment $-CH_2-CH_2-$ is present in addition to the propylene fragment $-CH_2-CH_2-$

CH_2–. This assumption is supported by the fact that signal 9 shows further splittings, which can be interpreted as two overlapping triplets. A value of 12 for *n* can be calculated from the spectrum (averaged molecular weight 2100).

The IR spectra of stationary phases A–F are generally very similar (Fig. 8) with the exception of the already mentioned carbonyl vibration at 1700 cm^{-1} in samples E and F. In the IR spectrum of C, splittings can be observed at 500 cm^{-1} that are not present in A, B, D, E and F.

Stationary phase G (Silar 9 CP) also contains cyanopropylsiloxane (Fig. 9), with $(CH_3)_3Si$ end-groups. In addition to the signals that appear in A and B there is a broad signal at 7.4 ppm in the ^1H NMR spectrum that could be assigned to a phenyl group. The IR spectrum of G shows the aromatic CH valence vibration at 3050 cm^{-1} and the out-of-plane vibrations at 700 cm^{-1} in addition to the CN vibration at 2250 cm^{-1}. In general, it can therefore be said that Silar 9 CP (G) is a partially phenylated cyanopropylsiloxane. From the integral of the NMR spectrum one obtains a ratio of the phenyl to the cyanopropyl groups of about 1:4; *n* is calculated to be 10 (averaged molecular weight 2080).

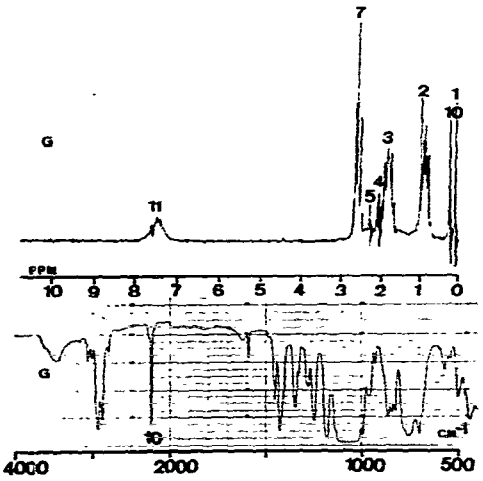


Fig. 9. ^1H NMR and IR spectra of Silar 9 CP as stationary phase.

Summarizing our results, we can confirm that the choice of Silar 10 C, SP 2340, Silar 9 CP or OV-275 as a liquid phase offers many advantages for the analysis of complex fatty acid methyl ester mixtures on packed columns and in capillary column GLC.

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