Separation of Petroselinic (*cis*-6 18:1) and Oleic (*cis*-9 18:1) Acids by Gas-Liquid Chromatography of Their Isopropyl Esters

Robert L. Wolff[a,*] and **Frederic F. Vandamme**[b]
[a]ISTAB, Universite Bordeaux I, 33405 Talence Cedex, France and [b]ISIH, 7800 Ath, Belgium

Petroselinic (*cis*-6 18:1) and oleic (*cis*-9 18:1) acids that occur together in Umbelliferae seeds can be resolved by gas-liquid chromatography (GLC) of their methyl or isopropyl esters on a 50 m × 0.25 mm fused-silica capillary column coated with a 100% cyanopropyl polysiloxane stationary phase (CP Sil 88). The use of isopropyl esters instead of methyl esters increases the difference between equivalent chainlengths from 0.06 carbon unit up to 0.08. This is sufficient to obtain an almost base-line resolution between the two components. *cis*-Vaccenic acid is completely separated from oleic acid in both derivative forms. GLC of fatty acid isopropyl esters on an appropriate capillary column thus appears to be the simplest means to simultaneously and accurately quantitate petroselinic, oleic and *cis*-vaccenic acids.

KEY WORDS: Gas-liquid chromatography, isopropyl esters, oleic acid, petroselinic acid, separation, Umbelliferae.

It has been repeatedly stated (1–5) that petroselinic (*cis*-6 18:1) and oleic (*cis*-9 18:1) acids that occur together in most Umbelliferae seed oils (6) are not readily resolved by gas-liquid chromatography (GLC) of their methyl ester derivatives. An accurate determination of petroselinic acid (a potential source of lauric and adipic acids) content in these oils is thus difficult, and time-consuming or complex analytical procedures have to be used. One of the most popular has been an oxidative cleavage of the ethylenic bonds in isolated monoenes followed by GLC of the resulting fragments (6–10). Clear-cut separations of petroselinic and oleic acids (or acid methyl esters) have been achieved by thin-layer chromatography on silver-impregnated alumina sheets (8). Derivatives other than simple methyl esters [trimethylsilyloxy (4,5,11) or epoxy (12) derivatives] of petroselinic and oleic acid methyl esters have been partially resolved by GLC-mass spectrometry. Another way to determine the petroselinic acid content in oils is to combine data obtained by GLC (sum of unresolved petroselinic and oleic acids) and by ^{13}C nuclear magnetic resonance (NMR) spectroscopy (ratio of oleic acid on petroselinic acid) (2,3).

However, it would appear that petroselinic and oleic acid methyl esters analyzed by GLC on cyanoalkyl polysiloxane stationary phases display slightly (but significantly) different equivalent chainlengths (ECL) (1,13,14). ECL data reported in the literature for these two fatty acids (Table 1) differ by 0.04 to 0.06 carbon units (1,13,14). Logically, these differences should be sufficient to obtain a more or less satisfactory resolution between the two isomers. In this report, we show that the use of a fused-silica capillary column coated with a 100% cyanopropyl polysiloxane stationary phase (CP Sil 88) allows an almost base-line resolution of petroselinic and oleic acids in Umbelliferae seed oils, provided these acids are analyzed as isopropyl ester derivatives.

EXPERIMENTAL PROCEDURES

Fatty acid standards and oil samples. Pure fatty acid methyl esters were purchased from Sigma Chemical Company (St. Louis, MO). These include 16:0, 18:0, 20:0, *cis*-6 18:1 and *cis*-9 18:1 acids. Common Umbelliferae seeds (parsley, carrot, fennel and celery) were purchased from local seed shops. Oil was extracted by crushing the seeds (5–10 g) in hexane and filtering the resulting suspension.

TABLE 1

Comparison of Equivalent Chainlength Values for Fatty Acid Methyl and Isopropyl Esters on Capillary Columns Coated with Cyanoalkyl Polysiloxane Stationary Phases

	ECL[a]				
	FAME				FAIPE
Fatty acid	(13)[b] Silar 10C[c]	(14) SP-2340	(1) CP Sil 84	(*) CPSil 88	(*) CPSil 88
cis-6 18:1	18.56	18.47	18.43	18.54 ± 0.01	18.37 ± 0.01
cis-9 18:1	18.61	18.53	18.47	18.60 ± 0.01	18.45 ± 0.01
cis-11 18:1	18.71	18.63	18.54	18.70 ± 0.01	18.56 ± 0.01
cis-9,*cis*-12-18:2	19.51	19.35	19.20	19.50 ± 0.01	19.24 ± 0.01
Δ(6–9)[d]	0.05	0.06	0.04	0.06	0.08
Δ(9-11)	0.10	0.10	0.07	0.10	0.11

[a]ECL, equivalent chainlength; FAME, fatty acid methyl ester; FAIPE, fatty acid isopropyl ester.
[b]References between parentheses. Asterisks correspond to values (means ± SD of 10 determinations) obtained in this study.
[c]Trade mark of the column.
[d]Differences between ECL values.

*To whom correspondence should be addressed at ISTAB, Laboratoire de Lipochimie Alimentaire, Université de Bordeaux I, Allee des Facultes, 33405 Talence Cedex, France.

Preparation of fatty acid methyl and isopropyl esters (FAME and FAIPE). FAME were prepared by transesterification of the oils with 14% BF_3 in methanol (wt/vol) according to Smith and Morrison (15). FAIPE were prepared starting with FAME or seed oils and reacting them with isopropanol in hexane solution with H_2SO_4 as a catalyst (16).

Purification of FAME and FAIPE. FAME and FAIPE were freed from volatile unsaponifiable components by thin-layer chromatography on silica-gel precoated plates (DC Vertigplatten Kieselgel H, Merck, Darmstadt, Germany). The migration solvent was hexane/diethyl ether/acetic acid (90:10:1, vol/vol/vol). FAME and FAIPE were then extracted from the silica-gel and further analyzed by GLC.

GLC. Analyses of FAME and FAIPE by GLC were carried out on a Carlo Erba 4130 chromatograph equipped with a flame ionization detector and a split injector (Carlo Erba, Milano, Italy). A fused-silica capillary column coated with 100% cyanopropyl polysiloxane (CP Sil 88, 50 m × 0.25 mm i.d., 0.20 μm film; Chrompack, Middleburg, Holland) was used with helium as carrier gas (inlet pressure, 1.3 kg/cm^2). The column was operated isothermally at 165°C. Both injector and detector were kept at 250°C. Quantitative analyses were performed with an SP 4290 integrator (Spectraphysics, San Jose, CA). Adjusted retention times were measured between solvent peak (frontal tangent intercept with baseline) and peak markers printed by the integrator. ECL were determined according to Ackman (17) with 16:0, 18:0 and 20:0 methyl or isopropyl esters as reference compounds.

RESULTS AND DISCUSSION

ECL of oleic, petroselinic, *cis*-vaccenic and linoleic acid methyl and isopropyl esters are shown in Table 1. These data have been established for each individual component by using authentic standards (except for *cis*-vaccenic acid) and a CP Sil 88 capillary column. The operating conditions (see Experimental Procedures section) were chosen to obtain the best resolution between petroselinic and oleic acid methyl or isopropyl esters analyzed as an approximately 1:1 (w/w) mixture. Under these conditions, an experimental value of 142,000 theoretical plates was calculated for oleic and linoleic acid methyl esters. This value is in good agreement with the supplier's data of 141,000 theoretical plates. ECL data are compared with values for methyl esters published in the literature (1,13,14). Our values for methyl esters differ by less than 0.02 unit from those determined with a Silar 10C capillary column by Scholfield (13). This is also true for linoleic acid. They systematically differ from values obtained on an SP-2340 column (14) by 0.07 ECL unit. This difference is independent of the ethylenic bond position. This difference is doubled for linoleic acid. Differences between ECL values determined on CP Sil 84 and 88 columns increase as the double bond moves away from the carboxylic end. The difference between ECL of petroselinic and oleic acid methyl esters on the CP Sil 88 column (0.06) is the same as that determined on an SP-2340 column (Table 1). As expected, this difference allows a fairly good separation of the two components in mixtures containing approximately equivalent amounts of petroselinic and oleic acids (Fig. 1). However, when petroselinic acid is the major component, a situation that occurs in most Umbelliferae seed oils (6), oleic acid can be partly fused with (although it remains distinguishable from) the trailing edge of petroselinic acid (Fig. 1). A similar partial separation was obtained by GLC of FAME trimethylsilyloxy derivatives on an SE 30 capillary column (4,5), except that petroselinic acid eluted after oleic acid on this column. With such poor resolutions, quantitation may be less accurate. It also has been claimed that a better resolution could be obtained with trimethylsilyloxy derivatives analyzed on a Carbowax 20M capillary column (5,11). However, a severe drawback is that the reactants used for silylation of FAME rapidly deteriorate the column (5,11).

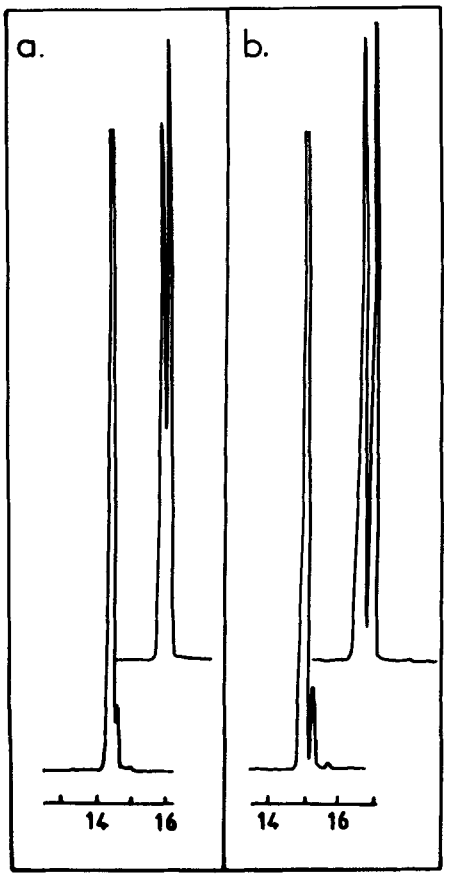

FIG. 1. Chromatograms of mixtures of methyl esters (a) or isopropyl esters (b) of petroselinic (first eluting peak) and oleic acids. Upper chromatograms: artificial mixtures of almost equivalent quantities of authentic petroselinic and oleic acid derivatives. Lower chromatograms: petroselinic and oleic acid derivatives prepared from celery seed oil. Analyses on a CP Sil 88 fused-silica capillary column. Adjusted retention times in min.

The best conditions of temperature and pressure to analyze isopropyl esters are the same as those used for methyl esters. This is apparently at variance with our previous observations (18) on the separation of linolenic acid geometrical isomers (LAGI). In this case, isopropyl esters had to be analyzed at higher pressure and temperature than methyl esters. In fact, the optimized conditions for the separation of octadecenoic acid methyl esters described in the present paper are not the same as those used for the best resolution of LAGI methyl esters (results not shown). Adjusted retention times for stearic and linoleic

TABLE 2

Fatty Acid Composition (determined by gas-liquid chromatography of their isopropyl esters on a CP Sil 88 capillary column) of Some Umbelliferae Seed Oils

Fatty acid	Carrot[a]	Parsley	Fennel	Celery
16:0	4.4 ± 0.1[b]	5.4 ± 0.4	4.3 ± 0.1	6.2 ± 0.1
18:0	0.9 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.9 ± 0.3
cis-6 18:1	66.1 ± 0.7	77.0 ± 0.5	78.9 ± 0.2	67.2 ± 0.2
cis-9 18:1	12.8 ± 0.5	6.2 ± 0.4	5.1 ± 0.1	6.8 ± 0.2
cis-11 18:1	0.8 ± 0.1	0.2 ± 0.2	trace[c]	trace
cis-9,*cis*-12 18:2	14.9 ± 0.2	9.6 ± 0.7	10.6 ± 0.1	17.9 ± 0.1

[a]Latin names and varieties, from left to right: *Daucus carota*, "rouge demi-longue Nantaise ameliorée"; *Petroselinum sativum; Foeniculum vulgare*, "Fino Zefa"; *Apium graveolens*, "Géant de Prague".
[b]Values are expressed as weight percentages relative to total fatty acids and are means ± SD of five analyses.
[c]Trace amounts, less then 0.2% of total fatty acids.

TABLE 3

Comparison of the Petroselinic and Oleic Acid Contents of Fennel (*Foeniculum vulgare*) Seed Oil by Different Analytical Procedures

Reference no.	*cis*-6 18:1[a]	*cis*-9 18:1	Analytical procedure
9	70.4	2.6	GLC//TLC/prep.GLC/O_3/GLC[b]
6	72.9	8.7	GLC//TLC/O_3/GLC
10	78.0	8.2	GLC//$KMnO_4$/GLC
3[c]	60.6	1.9	GLC//^{13}C NMR
	73.5	0	GLC//^{13}C NMR
2	77.9	7.7	GLC//^{13}C NMR
4	76.6	5.5	GLC//TMS/GLC
This study	78.9	5.1	GLC

[a]Weight percent relative to total fatty acids.
[b]GLC, gas-liquid chromatography; TLC, thin-layer chromatography; NMR, nuclear magnetic resonance; TMS, trimethylsilyl. GLC//TLC/prep.GLC/O_3/GLC, combination of data obtained by GLC of total fatty acid methyl esters (FAME) and GLC of aldehydes and aldesters obtained by ozonolysis of octadecamonoenoic acid methyl esters isolated by argentation-TLC and preparative GLC. GLC//TLC/O_3/GLC, data obtained by combining results of GLC of fragments generated by ozonolysis of monoenoic acid methyl esters isolated by argentation-TLC, and results of GLC of total FAME. GLC//$KMnO_4$/GLC, data obtained by combining results of GLC of fragments after cleavage with $KMnO_4$ and periodic acid of total fatty acids, and results of GLC of total FAME. GLC//^{13}C NMR, combination of data obtained by GLC of total FAME and ^{13}C NMR spectroscopy. GLC//TMS/GLC, combination of data obtained by GLC of total FAME and GLC on another column of trimethylsilyloxy derivatives of FAME. GLC, simple analysis of fatty acid isopropyl esters by GLC (no other complementary technique).
[c]Results given for two varieties.

acid methyl esters were 12.05 and 18.75 min, respectively. The corresponding values for isopropyl esters, run under the same chromatographic conditions, were 13.05 and 19.10 min. This means that isopropyl esters are slightly less volatile than the corresponding methyl esters, but also that interactions between ethylenic bonds and the stationary phase are slightly modified when methanol is replaced by isopropanol. Isopropyl esters of petroselinic and oleic acids have shorter ECL than their methyl ester counterparts (Table 1). Fortunately, the difference between isopropyl ester ECL is slightly enhanced (0.08) as compared with that occurring between the corresponding methyl esters. The separation factors increase from 1.012 to 1.016. Consequently, the resolution of the two components is significantly improved (Fig. 1). When fatty acid isopropyl esters prepared with Umbelliferae seed oils are analyzed, the resolution between the two components is still good and it allows a reliable quantitation of the two isomers (Fig. 1). The fatty acid compositions of four common Umbelliferae seeds obtained by using only GLC of FAIPE are given in Table 2. For one kind of seeds (*Foeniculum vulgare*), a comparison with results obtained by other authors is given (Table 3). This table clearly shows that a simple analysis by GLC of isopropyl esters is a fair alternative to other, more complex and time-consuming analytical procedures. In other instances we were able to separate two linolenic acid geometrical isomers (isopropyl esters of *cis*-9,*trans*-12,*trans*-15 and *trans*-9,*cis*-12,*trans*-15 18:3 acids) that were not previously separated as methyl esters (18). This means that some improvement can be obtained by changing the physicochemical properties of the solutes to be separated before changing the column. Perhaps a more systematic study of derivatives other than methyl esters could help in solving some delicate separation problems like that of petroselinic and oleic acids.

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