Determination of cyclopropenoic fatty acids by reversed-phase liquid chromatography and gas chromatography

ARTICLE *in* ANALYTICAL CHEMISTRY · DECEMBER 1983

Impact Factor: 5.64 · DOI: 10.1021/ac00264a025 · Source: PubMed

3 AUTHORS, INCLUDING:

Emile M Gaydou
Aix-Marseille Université
347 PUBLICATIONS **3,897** CITATIONS

Determination of Cyclopropenoic Fatty Acids by Reversed-Phase Liquid Chromatography and Gas Chromatography

Emile M. Gaydou,* Jean-Pierre Bianchini,[1] and Auguste Ralaimanarivo

Laboratoire de Phytochimie and Ecole Supérieure de Chimie de Marseille, Université de Droit, d'Economie et des Sciences, Centre Scientifique de Saint Jérôme, rue Henri Poincaré, 13397 Marseille Cédex 13, France

This article describes analyses in parallel by gas–liquid chromatography and high-performance liquid chromatography (GLC–HPLC) for quantitative analysis of cyclopropenoic fatty acid (CPEFA) methyl esters in oils. The separation of the two CPEFA (malvalic and sterculic) methyl esters from methyl oleate was carried out by HPLC using a reversed-phase column (RP-8). The mobile phase consisted in an acetonitrile–water (85:15, v/v) mixture and quantitation was obtained with an UV detector at 206 nm. The malvalate and sterculate vs. oleate ratio areas were used with oleate results obtained by GLC using packed or glass capillary columns for the calculation of fatty acid composition except for the cyclopropenoids. Samples of cottonseed and kapok and baobab seed oils were examined. This parallel GLC–HPLC method was compared to direct GLC and hydrobromic titration of CPEFA. It gives accurate results at concentrations as low as 0.15% of each CPEFA and requires less than 1 mg of oil for an analysis.

Malvalic (8,9-methylene-8-heptadecenoic) and sterculic (9,10-methylene-9-octadecenoic) acids (CPEFA) occur in cottonseed (*1*, *2*), kapok (*3*, *4*), and baobab (*5*) oils. These are consumed in quantity as edible oils.

Various methods have been proposed for the quantitation of CPEFA in oils and some have been reviewed by Coleman (*6*). At present, hydrogen bromide (HBr) titration is generally accepted as a good primary method for determining CPEFA content in cottonseed oils. Several authors have recently described improvements in the preparation of samples, in the titration procedure including use of the color indicator (*7*) or potentiometric procedure (*8*). These methods can yield accurate results, but only as total CPEFA content. The use of gas–liquid chromatography (GLC) for the determination of CPEFA has been proposed (*9*, *10*). Prior to GLC analysis, selective derivatization of CPEFA is needed because these compounds contain a cyclopropene ring and are unstable at high temperature. Coleman (*6*) found that the method of Raju and Reiser (*9*) was unsatisfactory and that the method of Schneider et al. (*10*) was only satisfactory at high CPEFA levels. The determination of CPEFA without previous derivatization was first reported by Recourt et al. (*11*), using on-column injection. More recently, Fischer and Schuller (*12*) and Bianchini et al. (*4*) using glass columns have attempted to quantitate CPEFA without previous derivatization. Bianchini et al. have observed a slight decomposition of CPEFA on a Carbowax 20M column at 170–190 °C (*4*). Fischer and Schuller have observed that the decomposition of methyl sterculate can occur even with glass columns (*12*).

The separation of CPEFA from saturated and mono-, di-, and triunsaturated fatty acid methyl esters has been achieved by using RP-8 or RP-18 high-performance liquid chromatography (HPLC) columns (*13*). The purpose of this work is to describe a new rapid assay of CPEFA, without previous derivatization, using parallel analyses by GLC–HPLC. Results obtained are compared with direct GLC using a BDS glass capillary column and HBr titration. This method was tested with olive oil containing various amounts of CPEFA and was applied to oil samples (cottonseed oil, peanut oil adulterated with cottonseed oil, and kapok and baobab seed oils).

EXPERIMENTAL SECTION

Samples of Seed Oils. Samples of refined olive and peanut oils were purchased from a local grocery. Cottonseeds (*Gossypium hirsutum*, Acala 1517 BR variety) were obtained in 1981 from the Compagnie Francaise pour le Développement des Fibres Textiles (CFDT, Mahajanga, Madagascar). Kapok seeds (*Ceiba pentandra*) were collected in the Antsiranana area (1980), together with baobab seeds (*Adansonia suarezensis*) (1981). Oils were extracted from crushed seeds with light petroleum (40–60 °C) by using a Soxhlet apparatus. The seeds were found to contain about 30% oil for cottonseed, 24% for kapok, and 35% for baobab.

Materials. Acetonitrile and methanol for chromatography were purchased from E. Merck (Darmstadt, GFR). The water employed for HPLC was double distilled in quartz. Malvalic (18:CE) and sterculic (19:CE) acids were obtained from kapok seed oil. The oil was transesterified with methanol by using sodium methoxide. The resulting methyl esters were subjected to a serie of urea clathrations and fractional crystallizations from methanol. The purification of the malvalate and sterculate concentrate was performed by a method similar to that described by White et al. (*14*). The final purity of methyl malvalate and methyl sterculate was higher than 99%, as indicated by GLC at 150 °C on a BDS glass capillary column.

Preparation of Methyl Esters. Methyl esters were prepared from oils by transmethylation with sodium methoxide as previously indicated (*4*).

High-Performance Liquid Chromatography. A Spectra Physics Model SP 8000 B HPLC instrument equipped with an UV Model SP 8400 ultraviolet detector was employed for the detection of fatty acid methyl esters. A Hibar (E. Merck) column (4.6 mm i.d. × 250 mm) slurry packed with LiChrosorb RP-8 (particle diameter 7 μm) was used as the RP sorbent. The experiments were performed at ambient temperature (20–22 °C) with detection at 206 nm. The mobile phase was an acetonitrile–water (85:15, v/v) mixture. The water content was determined by comparing the density difference of the solvent mixture and pure acetonitrile with a standard curve. Injections of 0.1% to 1% methyl ester solutions in acetonitrile were carried out with a 10-μL sample loop. The flow rate was set at 1.0 mL min^{-1}. Prior to injection, all sample solutions were filtered through a Millipore Fluoropore (PTFE) membrane filter of 0.5 μm pore size. Retention times and capacity factors were evaluated from the position of peak maxima on the chromatogram and the mobile phase hold-up time was obtained by injecting sucrose and measuring the retention time of the corresponding peak. Measurements of the capacity factors (k') were made in triplicate and

[1] Present address: Etablissement d'Enseignement Supérieur des Sciences Agronomiques, Département des Industries Agricoles et Alimentaires, B.P. 175, Antananarivo, République Démocratique de Madagascar.

0003-2700/83/0355-2313$01.50/0

Table I. Capacity Factors, Sensitivity, and Response Factors of Fatty Acid Methyl Esters[a]

fatty acid methyl ester				
carbon no.	name	k'	sensitivity, ng	RRF[b]
18:1	oleate	4.72	65.0	1.0
18:CE	malvalate	4.47	7.1	3.5
19:CE	sterculate	5.53	7.1	3.5

[a] Determined on RP-HPLC at 206 nm using a LiChrosorb RP-8 column and acetonitrile–water (85:15, v/v) as eluent. [b] RRF, relative response factor expressed as the ratio of the response factor of the compound under examination to the response factor of methyl oleate.

were reproducible within 3%. The sensitivity was determined as being the smallest amount of fatty acid methyl ester that gives a peak height twice the peak-to-peak noise level at 206 nm. The linearity of the responses for methyl oleate (18:1), methyl malvalate (18:CE), and methyl sterculate (19:CE) was determined by using four standard mixtures. Response factors determined at 206 nm were expressed against methyl oleate, which was chosen as internal standard.

Gas–Liquid Chromatography. An Intersmat IG 12 DFL (93 Pavillon-sous-bois, France) gas chromatograph equipped with a flame ionization detector and a glass injector was used for the analysis with the glass capillary column (20 m long, 0.4 mm i.d.) coated with butanediol succinate (BDS) (phase thickness 0.20 μm). Injection port, detector, and column temperatures were 220 °C, 200 °C, and 150 °C, respectively. The carrier gas was hydrogen and flow rate was 5 mL min^{-1} with a split ratio of 1/15. A Perkin-Elmer Model 56 recorder was used and peak areas were integrated by a Spectra Physics Minigrator integrator.

A Girdel Series 75 (92 800 Puteaux, France) gas chromatograph equipped with a flame ionization detector was used for the analysis with the DEGS column. The column, 10 ft × 0.125 in. o.d. stainless steel tubing packed with 10% stabilized DEGS on Chromosorb WAW 80–100 mesh, was operated isothermally at 165 °C. Injection port and detector temperatures were 280 °C and 260 °C, respectively. Nitrogen at a flow rate of 40 mL min^{-1} was the carrier gas. An Intersmat ICR 1 recorder integrator was used for the peak area determinations.

Hydrogen Bromide Titration of CPEFA. The quantitative determination of CPEFA with hydrobomic acid was done with the Durbetaki method (*15, 16*). With unrefined and unbleached oils the best results were obtained with a silver–silver chloride indicator electrode and a standard calomel reference as indicated by Zeringue and Feuge (*8*). In these last experiments 2.5-g portions of oils were used for the analysis and were titrated at 75 °C with 0.1 mol L^{-1} HBr. Results were expressed in sterculic acid (mol wt 294).

RESULTS AND DISCUSSION

Since CPEFA are known to be quite unstable at high temperature, we have tried to characterize and to quantitate them by a less rigorous HPLC procedure. The use in parallel of GLC for the accurate quantitation of common fatty acids and HPLC for the relative determination of CPEFA at low temperature, against methyl oleate, should represent a convenient method for the determination of the fatty acid composition of seed oils rich in oleate, with special reference to CPEFA. We have applied this method to the analysis of various oil samples containing CPEFA, i.e., cottonseed, kapok, and baobab seed oils.

Reversed-Phase HPLC of CPEFA. The use of acetonitrile–water solvent to obtain good separation of fatty acid esters has been improved by Scholfield (*17*). Studies of the capacity factors of various fatty acid methyl esters, including CPEFA and cyclopropanic fatty acid (CPAFA) methyl esters by reversed-phase HPLC using acetonitrile at various water contents (*13*), have shown that the most useful eluent system was acetonitrile–water (85:15, v/v). The sensitivity in HPLC analyses, depends upon numerous factors and is improved when the absorbance is higher at a particular wavelength. The sensitivity of saturated fatty acid methyl esters is low (233 ng at 195 nm and 287 ng at 205 nm for methyl heptadecanoate) showing therefore that the use of HPLC to quantitate saturated esters is not accurate. Oleic acid is always found in seed oils and since its capacity factor is between those of malvalic (18:CE) and sterculic (19:CE) acids, we have used this acid as internal standard for the quantitation of CPEFA. The wavelength of the ultraviolet detector was chosen to obtain the highest sensitivities for oleic, malvalic, and sterculic fatty acid methyl esters. The best results were obtained at 206 nm (Table I). The compound sensitivities were relatively high, therefore less than 1 mg of oil was needed for one analysis if the CPEFA content in oil was about 1%. The response factors for these three esters were determined by plotting their peak areas vs. concentration. The area responses were linear, and identical for malvalate (18:CE) and sterculate (19:CE) esters. The relative response factors expressed against methyl oleate are given in Table I.

Quantitative Analysis of Olive Oil Containing CPEFA. The determination of CPEFA in seed oils using analyses in

Table II. Fatty Acid Analysis[a] of Olive Oil Containing Various Amounts of CPEFA Esters

	added CPEFA[a]									
	18:CE[b] 0.0; 19:CE[c] 0.0		18:CE 0.6; 19:CE 0.7				18:CE 9.5; 19:CE 4.5			
	GLC			GLC	GLC-HPLC[g]			GLC	GLC-HPLC[g]	
fatty acid	BDS[d]	DEGS[e]	HPLC[f]	BDS	BDS	DEGS	HPLC[f]	BDS	BDS	DEGS
16:0	15.0	14.9		14.8	14.7	13.3		12.6	12.4	12.5
16:1	1.5	1.5		1.5	1.5	1.6		1.2	1.1	0.8
18:0	2.7	2.6		2.6	2.6	2.8		2.4	2.3	1.5
18:1[h]	65.8	66.0	93.5	65.0	64.8	62.4	54.2	57.0	56.0	55.7
18:2	14.1	13.5		14.3	14.2	16.6		14.5	14.3	15.1
20:0	0.5	0.8		0.4	0.4	0.8		0.4	0.4	0.3
18:3 + 20:1[i]	0.4	0.7		0.5	0.5	1.4		tr	tr	0.6
18:CE[b]			2.7	0.5	0.53	0.51	3.8	8.1	9.1	9.0
19:CE[c]			3.8	0.6	0.75	0.72	15.0	3.8	4.4	4.4
total CPEFA				1.1	1.28	1.23		11.9	13.5	13.4
recovered CPEFA				84.6	98.4	94.6		85.0	96.4	95.7

[a] Percent by weight. [b] 18:CE, malvalic acid (8,9-methylene-8-heptadecenoic). [c] 19:CE, sterculic acid (9,10-methylene-9-octadecenoic). [d] BDS glass capillary column at 150 °C. [e] DEGS packed column at 165 °C. [f] LiChrosorb RP-8 column. Relative composition for 18:1, 18:CE, and 19:CE. [g] Calculated from the relative composition found by GLC (without CPEFA on the BDS column) and the relative composition found by HPLC. [h] 18:1 is the sum 18:1 ω 9 + 18:1 ω 7 on the BDS column. [i] 18:3 and 20:1 have the same ECL on the DEGS column.

Table III. Fatty Acid Methyl Ester Analyses[a] of Cottonseed and Kapok and Baobab Seed Oils by GLC and Parallel GLC-HPLC Methods

	cottonseed oil				kapok				baobab			
			GLC-HPLC[d]				GLC-HPLC[d]				GLC-HPLC[d]	
fatty acid	GLC BDS[b]	HPLC[c]	BDS-HPLC	DEGS[e]-HPLC	GLC BDS[b]	HPLC[c]	BDS-HPLC	DEGS[e]-HPLC	GLC BDS[b]	HPLC[c]	BDS-HPLC	DEGS[e]-HPLC
14:0	0.8		0.8	0.9	0.5		0.5	tr	0.2		0.2	0.2
15:0					tr		tr	tr	0.3		0.3	0.2
16:0	24.1		24.1	23.2	18.1		17.7	17.9	45.1		44.9	47.9
16:1	0.8		0.6	0.6	0.7		0.7	0.5	0.6		0.6	0.8
17:0	tr		tr	tr	tr		tr	tr	0.2		0.2	0.2
17:1	tr		tr	tr	0.8		0.8	0.7	0.3		0.3	0.3
17:2	tr		tr	tr	0.9		0.9	tr	0.1		0.1	tr
18:0	2.6		2.6	2.4	1.1		1.0	0.9	4.6		4.6	4.4
18:1	17.1	88.6	17.1	17.9	19.5	28.6	19.1	20.3	24.6	41.2	24.5	24.5
18:2	53.3		53.2	53.6	45.9		44.8	44.9	11.2		11.2	9.3
19:CA[f]			0.7	0.4	0.6		0.6	0.4	1.8		1.8	0.6
20:0	tr		0.5	tr	tr		tr	tr	0.1		0.1	tr
18:3 + 20:1	0.1		0.1	tr	0.3		0.3	tr	1.1		1.0	1.2
22:0	tr		tr	tr	tr		tr	tr	0.2		0.2	0.5
18:CE[g]	0.3	5.2	0.29	0.30	8.7	53.9	10.3	10.9	4.6	29.4	5.0	5.0
19:CE[h]	0.2	6.2	0.34	0.35	2.9	17.5	3.4	3.5	4.5	29.4	5.0	5.0
total CPEFA	0.5		0.63	0.65	11.6		13.7	14.4	9.1		10.0	10.0

[a] Percent by weight. [b] BDS glass capillary column at 150 °C. [c] LiChrosorb RP-8 column. Relative composition for 18:1, 18:CE, and 19:CE. [d] Calculated from the relative composition found by GLC (without CPEFA on the BDS column) and the relative composition found by HPLC. [e] DEGS packed column at 165 °C. [f] 19:CA, dihydrosterculic acid. Equivalent chain length, 19.28 on BDS and 19.53 on DEGS. [g] 18:CE, malvalic acid; ECL, 17.92 on BDS. [h] 19:CE, sterculic acid; ECL, 18.93 on BDS.

parallel by GLC–HPLC was checked with an olive oil containing a known amount of added malvalate (18:CE) and sterculate (19:CE) esters. Peaks of methyl malvalate, methyl oleate (18:1), and methyl sterculate are well resolved using a RP-8 column and acetonitrile–water (85:15, v/v) as mobile phase.

For the GLC analysis we chose a stainless-steel column packed with diethyleneglycol succinate (DEGS), the classic substrate for the analysis of fatty acid esters. The peaks of methyl linoleate (18:2) and methyl dihydrosterculate (19:CA) overlap between 170 and 200 °C but they are separated at a temperature of 165 °C (ECL, 19.32 and 19.53 for 18:2 and 19:CA, respectively. Inspections of chromatograms at temperatures ranging from 160 to 200 °C showed that methyl malvalate (18:CE) and methyl sterculate (19:CE) are partially decomposed. The peak areas of CPEFA, that we obtained by GLC [$A_{CPEFA(GLC)}$] were determined by using HPLC as follows:

$$\frac{A_{CPEFA(GLC)}}{A_{CPEFA(HPLC)}} = \frac{1}{RRF}\frac{A_{18:1(GLC)}}{A_{18:1(HPLC)}}$$

where $A_{CPEFA(HPLC)}$ is the area of one CPEFA (malvalate, 18:CE, or sterculate, 19:CE) obtained by HPLC, $A_{18:1(GLC)}$ is the area of oleate obtained by GLC, and $A_{18:1(HPLC)}$ is the area of oleate obtained by HPLC, and RRF, the relative response factor of one CPEFA against oleate. Since the RRF value was the same for the two CPEFA (Table I)

$$A_{CPEFA(GLC)} = \frac{1}{3.5}\frac{A_{18:1(GLC)}}{A_{18:1(HPLC)}}A_{CPEFA(HPLC)}$$

Results obtained for two concentrations of 18:CE and 19:CE in olive oil are given in Table II. The total CPEFA content recovered by the parallel analysis method was between 95 and 96%. This parallel GLC–HPLC method of CPEFA determination was applied by using a glass capillary column coated with butanediol succinate (BDS) and the results obtained for the quantitation of CPEFA content were compared to the values obtained by GLC. As shown in Table II, the recovered CPEFA content ranged from 85% using the GLC method with a BDS column to 96–98% with the BDS–HPLC method. Within the experimental errors, concentrations calculated from manual measurements agreed with those calculated from areas supplied by the data system in the case of the parallel GLC–HPLC method. The slightly lower recovery of CPEFA content in the case of the GLC method using the BDS glass capillary column may be explained by a beginning of CPEFA decomposition on the BDS column as it was soon observed for a Carbowax 20M glass capillary column (*4*).

Parallel GLC–HPLC Analysis of Cottonseed, Kapok, and Baobab Seed Oils. The analysis of cottonseed oil fatty acid esters was investigated. A typical HPLC chromatogram is given in Figure 1. Good resolution of methyl malvalate (18:CE) and methyl sterculate (19:CE) from methyl oleate is obtained. This figure illustrates the need for an efficient RP-8 column to get usable resolution of CPEFA for the calculation of relative composition of these three esters. Areas of the two small peaks on the front and the back of the large oleate peak were determined assuming that the overlapping areas were not symmetrical. The accuracy of quantitation was checked by analyzing the same cottonseed esters with the BDS glass capillary column at 150 °C. Results obtained are given in Table III. These low CPEFA content (0.5–0.65%) have been reported for malagasy cottonseed varieties (*4*). The total CPEFA content was slightly higher with the parallel GLC–HPLC method than with the GLC method. The fact that the malvalate content is higher than that of sterculate using the BDS column (0.3 vs. 0.2%), although when using the RP-8 column a reverse proportion is obtained (0.30 vs. 0.35%), may be explained by the beginning of the overlapping areas of 18:CE and 18:0 (ECL, 17.92 and 18.0, respectively).

The accuracy of the quantitation was checked by analyzing peanut oils adulterated by known amounts of cottonseed oils. With a peanut oil containing 50% cottonseed oil, the parallel GLC–HPLC method gave acceptable accuracy for both malvalate and sterculate. Inspection of a chromatogram obtained from a peanut oil containing 10% cottonseed oil reveals that the CPEFA are overlapped by the shoulder and the tail of methyl oleate peak which represent about 40% of the fatty acid composition of the oil mixture. Therefore the lower limit of detection of CPEFA using this parallel GLC–HPLC method

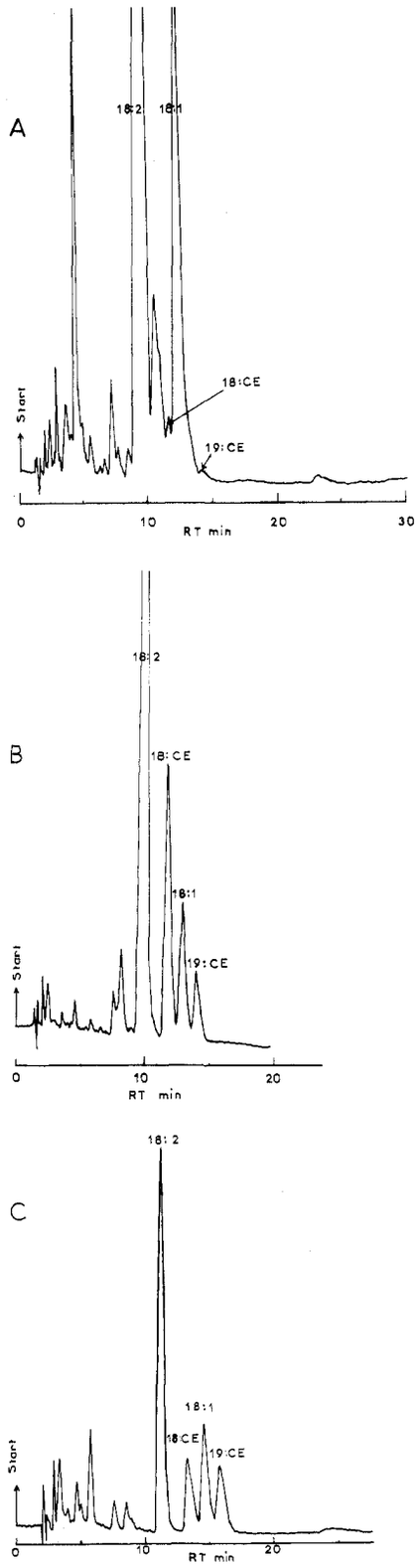

Figure 1. Chromatograms of seed oil fatty acid methyl esters on a LiChrosorb RP-8 column: solvent, acetonitrile–water (85:15, v/v); flow rate, 1.0 mL min^{-1}; UV detector, 206 nm. Peak identifications: 18:1, methyl oleate; 18:2, methyl linoleate; 18:CE, methyl malvalate; 19:CE, methyl sterculate; A (top), cottonseed oil; B (middle), kapok seed oil; C (bottom), baobab seed oil.

cannot be extended beyond concentrations of about 0.15%.

We have checked this parallel method by using samples of seed oils rich in CPEFA such as kapok and baobab seed oils. Chromatogram patterns obtained on RP-8 column are given in Figure 1. Quantitative analysis results are presented in Table III. In the case of kapok seed oil the main fatty acids are palmitic (18%), oleic (20%), and linoleic (45%) acids. The malvalic and sterculic acid contents obtained by the parallel GLC–HPLC method (10.3–10.9% and 3.4–3.5%, respectively) agreed with the results given elsewhere (*3*, *4*) and are higher than those given by GLC (Table III). Baobab seed oil analysis show that palmitic (45–48%), oleic (24%), and linoleic (9–11%) acids are the most important fatty acids. The malvalic acid content (5%) was found to be identical with that of sterculic acid, as shown in Table III. This fatty acid composition is in good agreement with previous results given for baobab oils (*5*).

Table IV. Comparison of Different Methods for CPEFA Quantitation[a] in Various Oil Samples

		seed oils/total CPEFA		
method	column phase	cotton-seed	kapok	baobab
HBr		0.67	14.2	10.1
GLC	BDS	0.5	11.6	9.1
GLC-HPLC	BDS-RP 8	0.63	13.7	10.0
	DEGS-RP 8	0.65	14.4	10.0

[a] Percent by weight.

Comparison of Different Methods of CPEFA Determination. The content of CPEFA in cottonseed and kapok and baobab seed oils was compared to the quantitative titration using hydrobromic acid with a silver–silver chloride indicator electrode, according to the method of Zeringue and Feuge (*8*). This method (1% level) gives values reproducible to about ±0.01 (*8*). As shown in Table IV, the CPEFA contents obtained by using direct GLC method with BDS are about 10% lower than those obtained with a GLC–HPLC or hydrobromic titration. The slight decomposition of CPEFA even with glass capillary columns was soon observed with Carbowax 20M at 190 °C (*4*) and with SP 2100 at 185 °C (*12*). The good agreement in the determination of methyl malvalate and methyl sterculate in oils obtained with hydrobromic titration supports the accuracy of the parallel GLC–HPLC method. Furthermore when using the parallel analysis method, certain advantages can be obtained: (i) less than 1 mg of oil is necessary for the analysis, therefore only one seed of the cotton plant, or less, is needed, (ii) the use of glass capillary columns is not necessary, (iii) this method yields individual concentrations for malvalic and sterculic acids. However this combined method is not accurate at lower CPEFA content (less than 0.15%) when oleic acid content is about 30–40%.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Compagnie Francaise pour le Developpement des Fibres Textiles (CFDT, Mahajanga, Madagascar) for cottonseed samples.

Registry No. Malvalic acid, 503-05-9; sterculic acid, 738-87-4; oleic acid, 112-80-1.

LITERATURE CITED

(1) Carter, F. L.; Frampton, V. L. *Chem. Rev.* **1964**, *64*, 497–525.
(2) Phelps, R. A.; Shenstone, F. S.; Kemmerer, A. R.; Evans, R. J. *Poult Sci.*, **1964**, *44*, 358–394.
(3) Berry, S. K. *Pertanika* **1979**, *2*, 1–4.
(4) Bianchini, J. P.; Ralaimanarivo, A.; Gaydou, E. M. *Anal. Chem.* **1981**, *53*, 2194–2201.
(5) Ralaimanarivo, A.; Gaydou, E. M.; Bianchini, J. P. *Lipids* **1982**, *17*, 1–10.
(6) Coleman, E. C. *J. Assoc. Off. Anal. Chem.* **1970**, *53*, 1209–1213.
(7) Feuge, R. O.; Codifer, L. P., Jr.; Zeringue, H. J., Jr., *J. Am. Oil Chem. Soc.* **1981**, *58*, 718–722.
(8) Zeringue, H. J., Jr.; Feuge, R. O. *J. Am. Oil Chem. Soc.* **1981**, *58*, 38–41.
(9) Raju, P. K.; Reiser, R. *Lipids* **1966**, *1*, 10–15.
(10) Schneider, E. L.; Loke, S. P.; Hopkins, D. T. *J. Am. Oil Chem. Soc.* **1968**, *45*, 585–590.
(11) Recourt, J. H.; Jurriens, G.; Schmitz, M. *J. Chromatogr.* **1967**, *30*, 35–42.
(12) Fischer, G. S.; Schuller, W. H. *J. Am. Oil Chem. Soc.* **1981**, *58*, 943–946.

(13) Bianchini, J. P.; Ralaimanarivo, A.; Gaydou, E. M. *HRCCC, J. High Resolut. Chromatogr. Chromatogr. Commun.* **1982**, *5*, 199-204.
(14) White, J. L., Jr.; Zarins, A.; Feuge, R. O. *J. Am. Oil Chem. Soc.* **1977**, *54*, 335-338.
(15) Durbetaki, A. J. *Anal. Chem.* **1956**, *28*, 2000-20001.
(16) Harris, J. A.; Magne, F. C.; Skau, F. F. *J. Am. Oil Chem. Soc.* **1963**, *40*, 718-720.
(17) Scholfield, C. R. *Anal. Chem.* **1975**, *47*, 1417-1420.

RECEIVED for review January 17, 1983. Accepted June 20, 1983. We thank the Fonds d'Aide et de Coopération (FAC) of France for a grant for A.R.

Selectivity in Pseudophase Liquid Chromatography

Daniel W. Armstrong*[1] and Gail Y. Stine

Department of Chemistry, Georgetown University, Washington, D.C. 20057

Pseudophase liquid chromatographic (PLC) separations (which make use of excluded volume effects) were evaluated for 14 compounds by using anionic micellar mobile phases and 8 compounds by using cationic micellar mobile phases. Most of the compounds studied were either dyes, alkaloids, or catalysts and all showed increased retention with increasing micelle content in the mobile phase. This retention behavior is the opposite of previously reported PLC work and consequently results in an unusual type of selectivity for this method. The mechanism, salt effects, and chromatographic usefulness of this technique are discussed.

Three of the more important goals in liquid chromatographic research are to improve selectivity, resolution, and detection. Pseudophase liquid chromatography (i.e., the use of micellar or cyclodextrin mobile phases) has been effectively used to improve both the selectivity (*1-8*) and detection (*9, 10*) of a variety of compounds while offering less for improved efficiency (*11*). Selectivity is improved because a solute can associate with the pseudophase through a combination of electrostatic, hydrophobic, and steric interactions (*1, 3, 6*). It is impossible for any homogeneous aqueous-organic or mixed organic mobile phase to achieve this range of interaction. Luminescence detection can be improved in PLC because many solutes show enhanced fluorescence (*9, 12-14*) and, in certain cases, room temperature liquid phosphorescence (*9, 10, 15*) when associated with micelles and cyclodextrins. In addition, many metal-dye complexes show increased absorbance in the presence of micelles (*16*).

The function of the pseudophase has been compared to that of the organic modifier in traditional reversed-phase LC. That is, an increase in the mobile phase concentration of modifier or pseudophase results in a decrease in the retention of the solutes being separated. This, however, is a greatly oversimplified view. The complexity of the interactions between a micelle and a solute, for example, can sometimes negate such simple comparisons. As will be shown in this work, compounds that do not bind to micelles can show highly unusual chromatographic behavior. In fact, all compounds can be placed in one of three classes based on their elution behavior with micellar mobile phases. Compounds that associate or bind to micelles show decreased retention when the mobile phase concentration of micelles is increased. Compounds that do not associate with micelles can show two different types of elution behavior. Their retention can be unaltered by the micelle content of the mobile phase (*nonbinding*) or their retention can increase with increasing micelle concentration (*antibinding*). True antibinding results from a compound being strongly excluded or repelled from the micelle.

As will be shown, separations that involve a combination of binding, nonbinding, and antibinding solutes give unique results. Furthermore, this distinctive chromatographic behavior can be used to elucidate a compound's hydrophobicity, hydrophilicity, and sometimes its ionization state.

EXPERIMENTAL SECTION

Materials. HPLC grade cetyltrimethylammonium chloride (CTAC) was obtained from Fisher. Electrophoresis purity sodium dodecyl sulfate (SDS) was obtained from BioRad Laboratories. Sodium 2-naphthalenesulfonate, sodium β-naphthoquinonesulfonate, 2-naphthol-6-sulfonic acid, blue tetrazolium, 2,3,5-triphenyl-2*H*-tetrazolium chloride, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide were obtained from Eastman Kodak. Ammonium thiocyanate, sodium nitroferricyanide, eriochrome blue SE, bromophenol blue (Na-salt), bromocresol green (Na salt), sodium chloride, and HPLC grade water were obtained from Baker. Ammonium molybdate, amaranth red, and alizarin red S were obtained from Fisher. Indigo carmine and sunset yellow were obtained from Pfaltz & Bauer. Tetraphenylphosphonium bromide and tetraphenylarsonium bromide were obtained from Alfa. Cinchonine from Sigma, naphthol green B from Allied Chemical, and *d*-turbocurarine chloride from ICN were also used as received. A 30 cm by 4 mm diameter Varian Micropak CN, 10 μm, alkylnitrile column was used for all LC measurements. Polygram polyamide-6 UV plates from Brinkman were used for all TLC measurements.

Methods. A Varian Model 5020 liquid chromatograph equipped with a UV 254-nm detector was used for all LC runs. The elution volumes of all solutes (vide supra) were determined at 22 °C using a pure aqueous mobile phase as well as at a variety of SDS concentrations (see Results and Discussion). Chromatographic "blanks" with aqueous NaCl solutions (up to 1.0 M) were run to show that the observed behavior was due to micelles and not to spurious salt effects. The total volume of the alkylnitrile column was 3.77 mL, the void volume (V_m) was estimated to be 1.75 mL, and the volume of the stationary phase (V_s) was estimated to be 2.02 mL. These were determined as previously reported (*3*).

All TLC developments were done in a $11^3/_4$ in. long, 4 in. wide, and $10^3/_4$ in. high developing tank. Development of all solutes was done by using mobile phases consisting of pure water (or salt water) and a variety of SDS and CTAC concentrations (see Results and Discussion). All SDS mobile phase concentrations must be corrected as previously indicated due to adsorption of SDS on the TLC plate during development (*7*). SDS critical micelle concentrations in the presence and absence of NaCl were measured with a Fisher (Model 20) Surface Tensiometer. All determinations were done at 22 °C on 20 mL of solution in a 15 cm diameter watch glass. One must be careful to use the correct critical micelle concentration when calculating partition coefficients in the

[1]Present address: Department of Chemistry, Texas Tech University, Lubbock, TX 79409.

0003-2700/83/0355-2317$01.50/0