Silicic Acid-Silver Nitrate Chromatography as an Enrichment Technique in Fatty Acid Analysis[1]

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Abstract

Silicic acid-silver nitrate chromatography as developed by De Vries (8,9) is a valuable technique for the quantitative determination of saturated fatty acids, and enrichment of fractions which permit the analysis of trace quantities of unusual fatty acids. Odd numbered fatty acids have been found in both saturated and monoene fractions of vegetable oils, such as olive, rapeseed and sunflowerseed. This technique coupled to oxidative degradation is useful for establishing the presence of minor quantities of positional isomers. Samples of purified polyunsaturated fatty acid esters are readily prepared using the technique as displacement chromatography.

Introduction

De Vries (8,9) developed silicic acid-silver nitrate column chromatography for the separation of the methyl esters of fatty acids into groups based on the degree of unsaturation and for the resolution of *cis-trans* octadecenoic esters. Padley et al. (1) extended the technique to the separation of glycerides on the basis of relative unsaturation. It was used in this laboratory to study the fatty acid composition of the oil from *Onosmodium occidentale* (3). The separation of saturated methyl esters was readily accomplished and the resultant enrichment showed the presence of odd numbered fatty acids which were not obvious from GLC charts of the original esters. In addition, the isomeric linolenic esters, 6,9,12 and 9,12, 15 were readily separated and purified by the appropriate choice of solvents.

The present investigation concerns further studies of silicic acid-silver nitrate column chromatography in the following areas: 1) quantitative determination of saturated fatty acids and detection of minor saturated fatty acids, 2) detection of isomeric mono- and dienoic fatty acids by the enrichment technique, and 3) preparation of pure unsaturated fatty acids by displacement chromatography.

Materials and Methods

The following vegetable oils were studied: rapeseed, olive, sunflower, coriander seed, *Lappula echinata* (bluebur), *Borago officinalis* (borage) and *Onosmodium occidentale* (western marble weed). Olive and sunflowerseed oils were commercial samples and the remainder were extracted from the ground seed with light petroleum ether (Skellysolve "F," bp 30–42C) using the Swedish steel tubes (6).

The methyl esters were formed by interesterification of the oil with 10 volumes methanol containing 0.5% (w/v) sodium methoxide. The mixture was refluxed for 90 min and the catalyst destroyed by the addition of acetic acid. Methanol was removed under reduced pressure in a rotary evaporator. The methyl esters were taken up in light petroleum ether, washed with water, dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure.

TABLE I

Quantitative Analysis of Saturated Acids in Vegetable Oils

Oil	% By wt		Composition[a] %					
	Column	GLC	14:0	15:0	16:0	17:0	18:0	20:0
Olive	16.4	16.6			82.8	0.8	16.4	
Sunflower	11.7	11.4	0.7	0.3	60.4	0.7	35.1	2.8
Coriander	4.2	4.0	0.8	0.8	72.3	0.8	22.1	3.2
Lappula echinata	8.1	7.8	0.4	0.4	71.9	1.1	26.2	
Borago officinalis	16.1	16.1	0.4		73.0	0.7	24.3	1.6
Onosmodium occidentale	8.7	8.7	0.1	0.1	65.8	0.9	32.4	0.7

[a] GLC analysis of saturated fraction from column.

The method outlined by De Vries (8,9) was followed for the preparation of the silicic acid-silver nitrate. Two columns were used: a) 25 mm x 400 mm length containing 50 g packing which could be used for 500 mg methyl esters; and b) 15 mm by 210 mm containing 20 g packing which could be used for 120 mg of methyl esters. The solvent systems were the same as used for the separation of the methyl esters of *Onosmodium occidentale* (3), saturated 10% benzene in light petroleum ether, monoene 40% benzene in light petroleum ether, diene 60% benzene in light petroleum ether and triene 5% ethyl ether in benzene. Ethyl ether was used to complete elution of the column where necessary. Samples were applied to the column in min quantities of light petroleum ether. Two hundred ml solvent were used to elute each fraction from the small column in the initial separations. Subfractionations on mono- and diene fractions were accomplished with 30 and 50% benzene in light petroleum ether, respectively.

The GLC analyses of the methyl esters were carried out using an *o*-phthalic ethylene glycol polyester on C-22 firebrick (2). The unsaturated esters and fractions separated by silicic acid-silver nitrate chromatography were oxidized by the permanganate-periodate method (5,7) and the resultant mono- and dicarboxylic acids analyzed as the decyl and methyl esters, respectively (4).

Results

A comparison between quantitative determination of saturated acid content and GLC analysis of the

TABLE II

Comparison of Fractions Obtained by Silicic Acid-Silver Nitrate Chromatography to Those Calculated from GLC Analysis

	Fraction			
	Saturated	Monoene	Diene	Triene
Solvent 200 ml.....	10 Benzene 90 Light petroleum ether[a]	40 Benzene 60 Light petroleum ether	60 Benzene 40 Light petroleum ether	95 Benzene 5 Ethyl ether
Column wt %.....	6.2	70.4	14.2	9.2
GLC %[b].....	5.6	69.7	16.4	8.3

[a] Skellysolve "F," bp 30–42C.
[b] Calculated from GLC analysis of mixed esters.

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[2] National Research Council of Canada Postdoctorate Fellow 1962–.

TABLE III

Composition of Rapeseed Fractions

Fatty acid	Fraction			
	Saturated	Monoene	Diene	Triene
12:0	0.1			
14:0	0.8			
15:0	0.4			
16:0	47.9			
16:1	....	0.4	0.1	
17:0	0.5			
18:0	23.4			
18:1	....	32.6	1.3	1.8
18:2	....		95.3	6.0
18:3	....			92.2
19:0	1.0			
20:0	14.2			
20:1	....	21.5		
20:2	....		3.3	
22:0	11.7			
22:1	....	45.5		

mixed esters on a polyester column was made by weighing the methyl ester fraction eluted from the silicic acid-silver nitrate column with 200 ml 10% benzene in light petroleum ether (Table I). Good agreement is found between the two methods, and GLC analyses of the saturated fraction show the presence of odd numbered fatty acids in significant amounts resulting from the enrichment effect.

Quantitative determination of saturated mono-, di- and triene contents by silicic acid-silver nitrate chromatography was tested using rapeseed oil methyl esters (Table II). GLC analyses of the eluted samples (Table III) show some mixing in the di- and triene fractions. The detection of odd numbered fatty acids in the saturated fraction is obviously due to the enrichment in this fraction.

The use of the enrichment technique for revealing minor constituents is shown in application to the oils from sunflowerseed, *Onosmodium occidentale* and *Lappula echinata* (Table IV). Odd numbered monoene fatty acids are present in oils from the latter two.

The enrichment technique was then extended to subfractionation of monoene fractions to test its effectiveness in revealing the presence of positional isomers within the fractions. Monoene fractions from olive, coriander and borage oils were rechromatographed and fractions were collected. The fractions were oxidized with the permanganate-periodate reagent and the fission products analyzed by GLC (Table V). The monoene fraction from olive oil shows 16:1, 17:1 and 18:1 as fatty acids, with enrichment of the 16:1 in the later fractions. Within the 18:1 fraction, the Δ11 isomer is concd in the first fraction. The proportion of C_7 dicarboxylic in the latter fractions indicates that the hexadecenoic acid contains both Δ7 and Δ9 isomers. Subfractionation of the monoene fraction from coriander and borage shows similar patterns. The analyses of monocarboxylic acids from oxidized fractions (Table VI) confirm the results in Table V.

TABLE IV

Separation of the Unsaturated Acid Methyl Esters in Oils by Silicic Acid-Silver Nitrate Chromatography

Fraction	Fatty acid	Composition of the fractions		
		Sunflower	*Onosmodium occidentale*	*Lappula echinata*
Monoene	16:1	0.3	1.0	1.1
	17:1	.....	0.6	0.4
	18:1	96.8	84.5	83.8
	19:1	.....	.....	0.6
	20:1	2.9	13.9	10.0
	22:1	.....	.....	4.1
Diene	18:2	98.9	98.2	100.0
	16:1	1.1	0.5	.....
	18:1	.....	1.3	

The subfractionation technique was then applied to the diene fraction obtained from the esters of sunflowerseed oil. The data (Table VII) indicate that no positional diene isomers are present. An increase in C_8 dicarboxylic acids in the later fractions was presumed to result from autoxidation of the methyl linoleate. Accordingly, the effect of autoxidation on the amount of C_8 dicarboxylic acid was studied by exposing a sample of methyl linoleate under atmospheric conditions at room temp. The progress of autoxidation was followed by peroxide values and UV absorption. Samples were oxidized and the products measured by GLC analysis. The results (Table VIII) show a progressive increase in C_8 dicarboxylic acid which is associated with increases in peroxide value and ultraviolet absorption. The elution of the original esters from an alumina column to remove peroxidized material resulted on some decrease in C_8 dicarboxylic acid and in peroxide value. A thin film of the oil which was partially polymerized also showed an increase in C_8 dicarboxylic acid. These results show conclusively that at least part of the C_8 dicarboxylic acid formed in the permanganate-periodate oxidation is due to autoxidation rather than overoxidation by the reagent.

The selectivity of silicic acid-silver nitrate chromatography for polyunsaturated esters indicated that pure samples of fatty acid esters could be rapidly prepared if the column was overloaded to permit retention of the desired ester. The addition of 4 g methyl esters of linseed oil in light petroleum ether to a column containing 10 g packing followed by washing with light petroleum ether yielded 0.3 g methyl linolenate (98.3% purity) which was eluted with benzene (Table IX). Similarly methyl linoleate was prepared from sunflowerseed oil in 0.2 and 1.7 g quantities with purities of 99.9 and 97.7%, respectively.

TABLE V

Enrichment of Monoenes

Oil and fraction	Wt mg	Fatty acid composition						Dicarboxylic composition						
		16:1	17:1	18:1	20:1	22:1	24:1	6	7	8	9	11	13	15
Olive														
1	140	0.5		97.9	1.6			0.9	0.6	1.1	87.0	10.4		
2	98	0.7		99.3				0.7	1.5	3.5	91.8	2.5		
3	52	1.8	0.4	97.8				0.9	1.8	1.3	94.2	1.8		
4	31	3.0	0.7	96.3				0.6	1.8	2.3	92.2	3.1		
5	21	4.7	1.0	94.3				0.6	1.8	2.2	94.3	0.9		
6	10	9.3	0.6	90.1				0.4	2.7	1.7	94.2	1.0		
7	3	33.7		66.3					7.6	1.0	89.7	1.7		
Coriander														
1	79	0.5		99.5				91.8		1.1				
2	39	0.5		99.5				82.3		1.4				
3	19	1.3		98.7				77.7		1.1				
4	9	4.9		92.6				72.8		2.0				
Borage														
1	8	....		41.4	28.1	21.3	9.2				38.8	34.4	17.6	9.2
2	11	1.3		85.2	9.6	3.9					84.9	12.4	2.7	
3	5	5.6		89.2	5.2						92.8	7.2		

TABLE VI

Monocarboxylic Acids of Some of the Fractions Recorded in Table V

Fat and fraction	Monocarboxylic acids mole %				
	6	7	8	9	12
Olive oil					
1	....	9.3	....	90.7	.....
4	....	3.2	0.9	95.9	.....
Coriander seed oil					
1	....	....	....	6.4	93.6
2	....	1.0	....	14.6	84.4
4	1.8	1.3	....	20.4	76.5

TABLE VII

Oxidative Studies of Linoleic Ester Fractions

Fractions	Wt	Dicarboxylic				Monocarboxylic	
		6	7	8	9	5	6
1	10	....	....	2.4	97.6	1.5	98.5
2	27	....	....	2.4	98.6	1.1	98.9
3	26	....	....	2.6	98.4	1.3	98.7
4	20	....	0.4	1.9	97.7	1.5	98.5
5	18	....	1.0	2.4	96.6	2.3	97.7
6	9	....	0.5	2.3	97.2	2.8	95.6
7	9	....	1.4	4.0	94.6	4.4	97.2
Linoleic		0.2	0.7	2.5	96.6	2.2	97.8

Discussion

The gravimetric determination of saturated acids by silicic acid-silver nitrate column chromatography using 100 mg samples of esters and 200 ml eluates of 10% benzene in light petroleum ether is readily accomplished. Further work in this laboratory has shown that petroleum ether (Skellysolve "B," bp 60–80C) is a preferred solvent if *trans* isomers of the monoene acids are present. These isomers would be eluted with more polar solvents, and GLC analyses would be required to demonstrate their presence in the saturated fraction.

The enrichment effect of fractionation by the silicic acid-silver nitrate chromatography shows the definite occurrence of odd numbered fatty acids in the saturated and monoene fractions of vegetable oils. The presence of odd numbered fatty acids has been shown previously in animal fats. Isovaleric acid has been cited as the singular instance for occurrence of other than even numbered acids in plant fats. The data in Tables I,II and IV show that C_{15}, C_{17} and C_{19} saturated and monoene acids occur in small proportions.

The enrichment effect used as a subfractionation technique and coupled to oxidative degradation amplifies the presence of positional isomeric acids in the monoene fractions. The data in Table V show that olive oil contains 7-hexadecenoic, 9-hexadecenoic, 9-octadecenoic and 11-octadecenoic acids as monoenes, and also a 9-heptadecenoic acid.

The oil from *Borago officinalis* contains 9-hexadecenoic, 9-octadecenoic, 11-octadecenoic, 13-eicosenoic, 13-docosenoic and 15-tetraceosenoic aids. The detection and estimation of the latter two minor constituent acids is very doubtful by direct GLC analysis, but is accomplished on the enriched chromatographic fraction.

TABLE VIII

Effect of Autoxidative on Linoleic Acid

Time (days)	$E^{1\%}_{1cm}$	Peroxide value	Dicarboxylic acids			
			7	8	9	10
Oil	.....	9.0	....	2.0	98.0	
Esters[a]	3.9	23.4	....	4.2	95.8	
0[b]	1.8	5.5	....	3.0	97.0	
5	146	1320	....	3.9	96.1	
8	271	2768	0.9	5.4	93.7	
13	217	2813	1.7	8.1	90.2	
43	.....	.....	1.7	11.7	86.6	
Esters[a]						
13	.....	2955	2.1	12.0	85.9	
Oil[c]	.....	.....	4.2	15.8	63.6	2.8

[a] As prepared from glyceride oil.
[b] After elution from alumina column to reduce peroxide content.
[c] Thin film exposed to atmospheric conditions, partially polymerized.

Application of the same technique to the diene fraction of sunflowerseed oil leads to the conclusion that this oil contains only one diene which is the 9,12-octadecadienoic acid. The study of monocarboxylic oxidation products shows a progressive increase in *n*-valeric acid which parallels the increase in C_8 dicarboxylic acid. The occurrence of the C_8 dicarboxylic appears to be due to autoxidation prior to analysis which is substantiated by the study on the effect of autoxidation as measured by peroxide values and spectral analysis on the content of C_8 dicarboxylic acid (Table VIII). These results show the necessity for using fresh samples of oils for studies on fatty acid composition.

Application of silicic acid-silver nitrate chromatography as a displacement technique facilitates the ready preparation of relatively pure samples of unsaturated acids in amounts from mg to g lots. The use of relatively large amounts of esters to flood the column and selection of solvents allows the column to retain the desired ester at max capacity of the column. It seems reasonable to expect that a scale up should be possible in order to produce larger quantities of purified esters and the technique offers the advantage of rapid preparation which avoids degradation.

Silicic acid-silver nitrate chromatography greatly facilitates the determination of fatty acid compositions of oils and fats. Since it separates on the basis of degree of unsaturation in the esters, it should find applications in determining fatty acid composition of complex mixtures and in demonstrating the presence of trace amounts of isomeric fatty acids.

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TABLE IX

Preparation of Purified Fatty Acid Esters

Acid	Source	Washing solvent	Elution solvent	Packing g	Esters g	Yield g	Purity
Tetraene	Bluebur	.....[a]	Ether	50	0.5	.090	100
Linolenic	Linseed	light petroleum ether[b]	Benzene	10	4	.300	98.3
Linoleic	Sunflower	light petroleum ether	40% Benzene	50	10	1.7	97.7
Linoleic	Sunflower	10% Benzene 90% light petroleum ether	40% Benzene	10	2	.2	99.9

[a] 5% Ether/95% benzene.
[b] Skellysolve "F," bp 30–42C.