Silver Nitrate-High Performance Liquid Chromatography of Fatty Methyl Esters

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ABSTRACT

Long chain fatty methyl esters have been separated by high performance liquid chromatography on the basis of number, position, and geometric configuration of double bonds with a silver nitrate-silicic acid column and benzene solvent. Saturated esters are eluted first, followed by methyl elaidate and then methyl oleate. Geometric isomers of methyl 9,12-octadecadienoate and of methyl 9,15-octadecadienoate are also well separated. Methyl linolenate is retained strongly on the column and its elution has not been observed, but the *trans,trans,trans* and *trans, cis,trans* isomers are separated.

INTRODUCTION

Chromatographic methods based upon complexing ability with silver ion have been widely used to separate long chain compounds on the basis of number and geometric configuration of double bonds. These methods fill a need not met by reverse-phase chromatography (1-6), where separations are also affected by other properties. We have used silver ion on a macroreticular sulfonic acid resin for methyl esters (7,8) and recently have found distinct improvement with a newer resin of increased surface area (9). Houx et al. (10,11) and Warthen (12) used similar procedures with different resins to separate geometric isomers of alkenol acetates and methyl esters. Lam and Grushka (13) used silver aluminosilicate to separate p-bromophenacyl esters; but, unlike the preceding methods, they report separation of positional isomers. With two exceptions (11,13) these techniques are best suited for small scale preparations rather than rapid analysis of small samples. Also, some of the macroreticular resins used have not always been readily available.

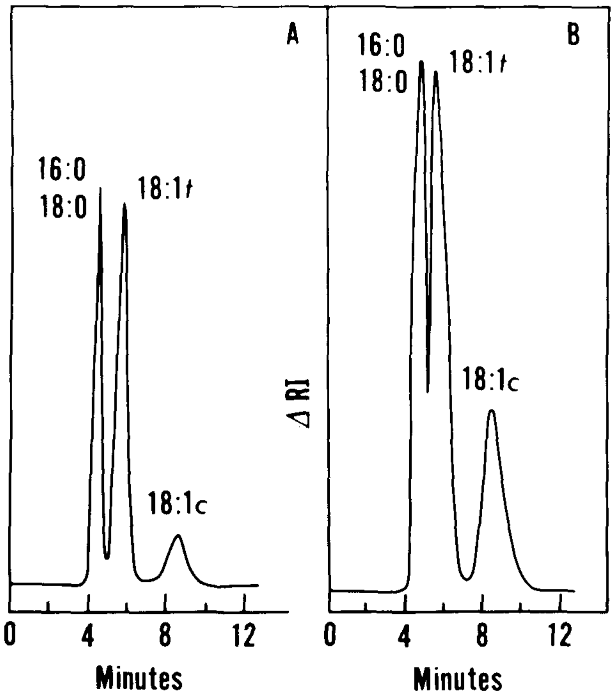

FIG. 1. Chromatograms of methyl palmitate, stearate, elaidate, oleate mixture on 2 mm i.d. x 61 cm $AgNO_3$-silicic acid column with 0.5 ml/min benzene. A, 0.5-μl sample RI 32X; B, 16-μl sample RI 128X nonlinear.

Silver nitrate-silicic acid mixtures have been even more widely used both in column and in thin layer chromatography since they were proposed by deVries (14) and by Morris (15). However, the necessity to use gradient elution and solvents which extract silver nitrate from the packing have prevented use of a differential refractometer to monitor eluants and have made reuse of columns difficult. Recently, Heath et al. (16,17) avoided these difficulties by using benzene as solvent with silver nitrate-silicic acid for high performance liquid chromatography (HPLC) of alkenol acetates.

We have investigated the use of HPLC with silver nitrate-silicic acid and benzene for methyl ester separations and compared it with our sulfonic resin method (9).

EXPERIMENTAL PROCEDURES

Silicic acid was coated with silver nitrate in a manner similar to that described by Heath et al. (16). In a 100-ml round bottomed flask, a solution of 2 g silver nitrate in 50 ml dry acetonitrile was added to 10 g of 24-44 μm Biosil A (BioRad Laboratories, Richmond, CA). Solvent was removed under vacuum at 60 C on a rotary evaporator. A 2-mm i.d. x 61 cm stainless steel column with 10 μm fitting at the detector end was filled with 1.85 g of the packing. Samples were run on a Waters ALC 202 instrument with benzene as eluant. Most chromatograms were run with a solvent flow of 0.5 ml/min at a pressure of ca. 380 psi. Samples were injected through a septum as a 20 volume percent solution in benzene.

Two 7 mm i.d. x 61 cm columns in series were also used for larger samples with a solvent flow of 5 ml/min.

RESULTS AND DISCUSSION

Many runs were made with a mixture of methyl palmitate, stearate, elaidate, and oleate with different flow rates and sample sizes. Hexane was added to some samples as an indicator of Vn, the retention volume of an unretained compound. Representative curves are shown in Fig. 1. Resolution between saturates and elaidate changes little up to a sample size of 4 μl but decreases for an 8- or 16-μl sample as shown in 1B. Average retention volumes calculated from retention time and flow rate are listed in Table I for a number of esters. For some esters where 11 to 28 runs were made, standard deviations are included. Retention volumes vary slightly from column to column. With a similar column, saturates and elaidate had the same retention volumes as in Table I, but retention volume for oleate was 4.78 ml and for linoleate, 9.80 ml.

A comparison of chromatographic parameters (18) calculated from data in Figure 1A and Table I, with values calculated for 100-200 mesh XE-284 from data in a previous publication (9), is shown in Table II. Although the values are very similar, because of the shorter elution times the HPLC method is much superior in plates generated per unit time. The shorter elution times with the HPLC method

TABLE I

Retention Volumes on 2 mm i.d. x 61 cm $AgNO_3$-Silicic Acid Column with Benzene Solvent

Compound	Retention volume, ml	Standard deviation
Hexane	1.94	0.04
Methyl palmitate, stearate mixture	2.26	0.07
Methyl arachidate	2.22	
Methyl laurate	2.50	
Methyl elaidate	2.84	0.11
Methyl oleate	4.30	0.12
Methyl linolate	8.00	
Methyl *cis*-9,*trans*-12 octadecadienoate	5.12	
Methyl *trans*-9,*trans*-12 octadecadienoate	3.56	
Methyl *cis*-9,*cis*-15 octadecadienoate	23.15	
Methyl *cis*-9,*trans*-15 and *trans*-9,*cis*-15 octadecadienoates	10.45	
Methyl *trans*-9,*trans*-15 octadecadienoates	5.35	
Methyl *trans*-9,*trans*-12,*trans*-15 octadecatrienoate	6.43	
Methyl *trans*-9,*cis*-12,*trans*-15 octadecatrienoate	8.75	

TABLE II

Chromatographic Characteristics of 100-200 Mesh Amberlite XE-284 and Silver Nitrate-Silicic Acid HPLC Columns for Methyl Oleate and Elaidate

Column	18:1 *trans*		18:1 *cis*		
	H,[a] cm	k'[a]	H, cm	k'	α[a]
100-200 mesh, XE-284 (9)	0.17	0.44	0.20	1.26	2.88
HPLC, $AgNO_3$-SiO_2	0.16	0.46	0.19	1.22	2.65

[a] H = height equivalent to a theoretical plate, k' = capacity factor, α = relative retention (18).

TABLE III

Area Percentages from Figure 1a Compared with Values by Other Methods

Chromatographic method	Composition[a] %		
	Saturates	Elaidate	Oleate
HPLC, $AgNO_3$-SiO_2	35	53	13
Column, XE-284 resin	32	55	12
Gas	33[b]	— 67	—
Capillary gas	—	55	—

[a] Infrared analysis indicated 50% elaidate.

[b] 28.7% palmitate, 4.7% stearate.

(ca. 9 min vs. 40 min for oleate and 16 min vs. 3.5 hr for linoleate) and the smaller sample size (0.5 µl vs. 20 µl) are advantageous for analytical purposes. Area percentages from Figure 1A are compared in Table III with values by other methods.

As seen in Table I, saturates are retained slightly after the hexane peak. This differs from XE-284 resin on which hexane is retained slightly longer than stearate and palmitate. As with XE-284, methyl arachidate has the same retention volume as stearate and palmitate and the value for methyl laurate suggests that short chain esters may be retained slightly longer.

Geometric isomers of methyl linoleate are well separated, but the all *trans* isomer is eluted only slightly before oleate. Similarly, geometric isomers of the methyl 9,15 octadecadienoate series are well separated but overlap the 9,12 series. Positional isomers of the 9,12 and 9,15 series are also separated. The all *cis* methyl linolenate was never seen to elute from the column, but retention volumes for two methyl octadecatrienoates are included in the table.

As a preparative technique, samples up to 100 µl were chromatographed on the larger 7-mm i.d. column with a flow rate of 5 ml/min. Retention volumes were: saturates 47 ml, elaidate 58 ml, oleate 90 ml, methyl 9-*cis*, 12-*trans* oatadecadienoate 113 ml, and linoleate 177 ml. Again retention times were shorter than on the SE-284 column, but maximum samples size was less (9).

More than 50 chromatograms were run on the original column before it was discarded because of poor resolution and baseline performance. This lifetime is satisfactory for practical use, but it is not as good as for silver resin columns, which in our experience last indefinitely. We did not find it necessary to wash our eluates with salt solution to remove silver as reported by Heath et al. (10). However, on occasion, with columns giving poor baselines, immiscible material giving a precipiate with hydrochloric acid has been found in the eluate flask.

Both silver nitrate-silicic acid HPLC and silver resin chromatography are capable of giving good separations of methyl esters. The HPLC procedure provides for faster separations with smaller samples and is useful for analytical work. Also, the materials for making silver nitrate-silicic acid columns are readily available, whereas the macroreticular resins have sometimes been in short supply. On the other hand, the silver resin separation is suitable for larger samples, it does not require high pressure apparatus, and the columns have been found more stable for long use.

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