Separation of Fatty Acid Methyl Esters on the Basis of the Number of Double Bonds Using Alumina Plates[1]

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ABSTRACT

The separation of fatty acid methyl esters (FAME) on the basis of the number of double bonds present in the molecule was accomplished using thin layer chromatography (TLC) on alumina. Hexane was used as the solvent in a continuous development mode using modified tanks. The separations were verified by gas liquid chromatography (GLC) and retention of structural integrity was confirmed by infrared analysis for *trans* isomerization and by ozonolysis-GLC for double bond position.

INTRODUCTION

The lipid chemist frequently is required to establish the identity of a fatty acid corresponding to a particular peak obtained by gas liquid chromatography (GLC). In addition to chain length, the number of double bonds present and their location is needed. The number of double bonds has usually been established by means of silver nitrate impregnated thin layer chromatography (TLC) plates (1,2). While excellent separations are achieved by this method, the prepared plates are not stable and are best used on the day of manufacture. They are also inconvenient to prepare and the dramatic rise in the price of silver has made this an expensive technique. Recently, while developing procedures for the separation of brominated from nonbrominated fatty acid methyl ester (FAME), it was observed that normal FAME could be efficiently separated on alumina TLC plates. This paper describes the techniques involved.

EXPERIMENTAL PROCEDURES

Materials

Standards, both single compounds and mixtures, were obtained from Nu Chek Prep, Elysian, MN. Alumina G TLC plates (500 micron) were from Analtech, Newark, DE. The pH of 1.06 g of alumina, taken from a plate and ultrasonically suspended on 25 mL of distilled deionized water, was 5.5. Also obtained from the same source were experimental PRE-adsorbant alumina G plates (250 micron). Other manufacturers' products that were evaluated and found satisfactory were: E.M. Reagents, Elmsford, NY, alumina on aluminum metal (200 micron) and J. T. Baker, Phillipsburg, NJ, alumina on glass (250 micron); both lack the capacity of 500 micron plates. One was found not to be satisfactory: E.M. Reagents, Elmsford, NY, alumina on glass (250 micron). Normal hexane, bp 67-69 C, was redistilled from all glass apparatus and 50 mg/L butylated hydroxy toluene was added as an antioxidant for the FAME (3). Rhodamine 6G, as a 0.05% solution in methanol, was used as a spray reagent. Other solvents and chemicals were either reagent grade or distilled in an all-glass apparatus.

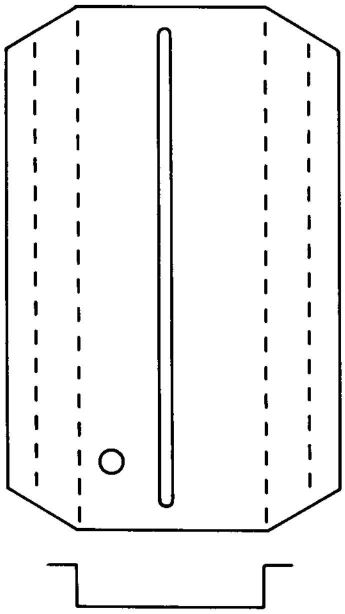

FIG. 1a. Layout pattern for aluminum TLC covers. Dashed lines indicate bending lines; bottom view shows finished cross-section from end. The round hold is for solvent addition and is normally plugged.

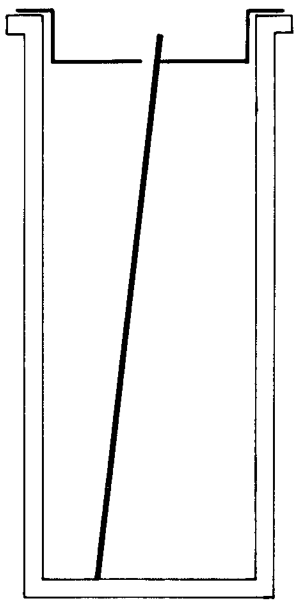

FIG. 1b. Cross-section of tank in use.

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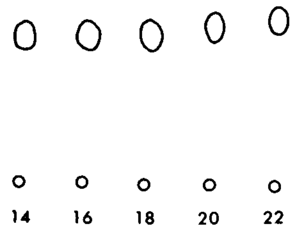

FIG. 2. Migration of 14-22 carbon chain monenes of FAME, 25 μg/spot, hexane as the developing solvent. Numbers designate number of carbons in chain.

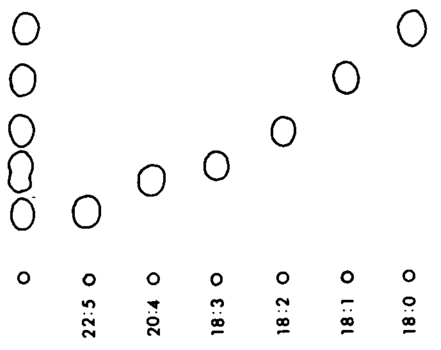

FIG. 3. Separation of FAME by number of double bonds on alumina G TLC plate. Mixture on left, remaining are single compounds—22:5 being 22 C, 5 double bonds, etc.

Equipment

Standard moulded glass TLC tanks were equipped with aluminum covers to replace the usual glass plates. The covers were made with a 0.6 by 21.6 cm (¼ by 8½ in.) slot in the center of the lid to permit continuous development and give support to the TLC plate. Figure 1a shows the layout before and end view after bending. It must be low enough in the tank to permit ca. 0.5 cm of the plate to protrude through the slot, thus allowing solvent evaporation from that edge. Figure 1b is a cross-section of the tank, cover and a plate. The use of a temperature controlled box for developing the chromatogram can be advantageous and was used on occasion. However, it should be pointed out that using hexane in a completely closed box can create explosive conditions even at 30 C. Sufficient circulation must be provided to keep the hexane/air concentration below the explosive level.

Alternatively, a glass tank was wrapped with four coils of rubber tubing and connected to a circulating water bath. Sheets of foamed polystyrene insulation were placed outside the tubing. This proved to be equally as satisfactory as the heated box and considerably safer. Separations obtained at 35 C in one hour were equivalent to those requiring 3 hr at room temperature.

Levels of *trans* isomerization were determined by AOCS method Cd 14-61 (4) and ozonolysis-GLC was carried out by the methods of Stein and Nicolaides (5), and Privett and Nickell (6).

RESULTS AND DISCUSSION

Since alumina has a lower capacity than silica gel, the permissible sample loading was determined using methyl oleate. To achieve the desired separations a maximum capacity of 25 μg/spot was established and used for all subsequent work. Similarly, a 2-hr development time was used throughout this study. Development times up to 4 hr were examined and they permitted increased separation of component centers; however, there was a concurrent increase in spot size.

The high resolution obtained with the system introduces other potential complications. Steadily increasing migration occurs with increasing chain length of a series of monenes (Fig. 2). Consequently, it is possible to have overlapping of specific unsaturation bands if chain length is quite variable in adjacent bands. Fortunately, few natural systems have such mixtures.

A separation of standards, 25 μg/spot for individual spots and 5 μg/spot for the collective spot on the left, is given in Figure 3. Resolution of all the samples by the number of double bonds present was accomplished. If necessary, resolution can be improved by reducing the sample size and still provide adequate material for subsequent ozonolysis and/or GLC.

A standard mixture of FAME containing equal weights of five fatty acids was developed at 26 C, dried under nitrogen, and the bands detected with Rhodamine 6G and ultraviolet light. Narrow strips between each band were removed and discarded to minimize any overlap. The FAME bands were then removed, eluted with ether, and subjected to GLC with the results given in Table I. Percent purity was

TABLE I

	% Purity in bands				
Band No.:	0	1	2	3	% Recovery[a]
16:0	49.0				100.0
18:0	50.9	0.2			103.9
18:1		99.1	0.9		81.5
18:2		0.7	95.0	1.5	79.0
18:3			4.0	98.5	58.7

[a] Recovery defined as peak area/peak area of 16:0 × 100. Samples were made to a known volume and specific volumes injected. Band numbers start from the top of the plate and correspond to the prevalent number of double bonds.

maintained at 95 % or greater by this technique, indicating its suitability for the uses suggested. All work reported was carried out on Analtech 500 micron plates. The 250 micron plates of the other two firms lacked the capacity desired. The experimental PRE-adsorbant 250 micron plates from Analtech gave superior separations over all others; however, they are not a stock item and are of lesser loading capacity.

Several papers (7-10) have described or referred to the use of alumina for lipid separations, usually as the ester of a short-chain alcohol. Alumina of basic pH is generally credited with hydrolysis or saponification of esters and strongly acidic adsorbants can cause a number of undesirable reactions (11). A pH of 5.5 is in an acceptable suggested range for adsorbants for lipids and the Analtech plates were examined for possible isomerization and hydrolysis. Within the detection limits of AOCS method Cd 14-61, no *trans* double bonds could be found in samples of methyl linoleate and linolenate that had been developed for 2 hr, removed as a band and extracted. Similarly, when a sample of methyl linoleate was streaked, developed and extracted, it gave retention times and peaks following ozonolysis identical to a sample not subjected to alumina TLC, indicating no migration of the double bonds during TLC. Hydrolysis of the ester bond did not appear to be occurring at any significant level since there was no evidence of the formation of the free acid and the recovery of applied standards was acceptable. The more refined alumina now available for chromatography, with pH and metal contamination controlled, may account for the improved performance of this adsorbant with FAME.

The possibility of using this procedure for the separation of triglycerides (TG) was briefly examined. The increased polarity exhibited by the terminal functional groups in TG compared to that of FAME precludes any possibility of separation with the solvent system described. Using a more polar solvent in order to increase TG migration would completely mask the small differences in polarity displayed by the number of double bonds present.

The described methodology provides an easy, convenient and economical alternative to the use of silver nitrate for the separation of FAME on the basis of the number of double bonds.

ACKNOWLEDGMENTS

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