

Large-scale separation of fatty acid methyl esters by column chromatography on acid-washed Florisil impregnated with silver nitrate

ROBERT L. ANDERSON and EDWARD J. HOLLENBACH
The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio

SUMMARY Rapid, large-scale separation of fatty acid methyl esters has been accomplished with acid-washed Florisil impregnated with silver nitrate. Recovery of material was

quantitative and the peaks contained only one type of fatty acid; i.e., saturated, monoenoic, or dienoic acid.

KEY WORDS fatty acid methyl esters · column chromatography · silver nitrate-impregnated Florisil · degree of unsaturation

SILVER NITRATE-IMPREGNATED silicic acid has been used to separate fatty acid methyl esters according to their degree of unsaturation and the geometrical configuration of their double bonds by both column chromatography (1) and thin-layer chromatography (2). Either of these methods is applicable when small quantities of methyl esters are to be resolved but when gram quantities of methyl esters are to be separated neither of the above techniques is satisfactory; the thin-layer chromatography because of its limited capacity and the column chromatography because the slow flow rate results in a long exposure of the esters to the adsorbent.

Carroll (3) has reported that extensive acid washing of Florisil results in a coarse (60–100 mesh) silicic acid which can be used to fractionate lipids. The present report describes the use of acid-washed Florisil impregnated with silver nitrate to separate large quantities of fatty acid methyl esters according to their double-bond content.

Acid-washed Florisil prepared as described by Carroll (3) was impregnated with silver nitrate by heating 100 g of the acid-washed Florisil in 200 ml of a 50% aqueous solution of silver nitrate at 100° for 30 min as described by De Vries (1). The impregnated Florisil was cooled, filtered, and dried at 120° for 16 hr. No attempt was made to determine the amount of silver nitrate retained by the Florisil. This treatment resulted in a white, coarse adsorbent. To prepare the adsorbent for packing the column, 100 g was suspended in 200 ml of hexane and heated on a steam bath for 5 min. When the slurry had cooled, it was added to a 70 × 1.9 cm column in small portions with gentle tapping of the column to aid packing

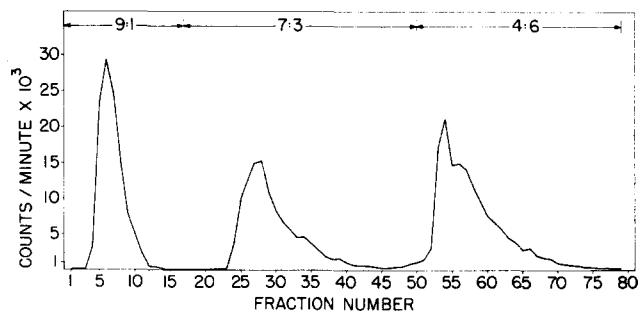


FIG. 1. Separation of fatty acid methyl esters on silver nitrate-impregnated acid-washed Florisil. Fractions were 50 ml. The solvent systems were hexane-benzene in the ratio indicated. Radioactivity is expressed as cpm/0.02% of each 50 ml of eluate measured in an end-window proportional counter.

TABLE 1 WEIGHT OF COMBINED FRACTIONS FROM COLUMN

Sample	Weight	Radioactivity
	mg	mc ¹⁴ C
Original	1807	10.03
Fractions		
1-4	26	0.07
5-10	571	2.64
11-22	28	0.11
23-40	543	3.10
41-50	60	0.25
51-75	575	3.80
76-79	3	0.02
Total Recovery	1806	9.99

of the adsorbent. In the separation described below, 1.8 g of uniformly labeled fatty acid-¹⁴C methyl esters, obtained from *Penicillium javanicum* (4), were fractionated on a column containing 100 g of the impregnated Florisil. The column was cooled to about 10° by a water-jacket and protected from light by a black cloth throughout the fractionation. The fatty acid methyl esters were eluted with increasing concentrations of benzene in hexane as shown in Fig. 1. The column eluate was collected in 50-ml fractions. The solvent was removed at 50° under reduced pressure and the residues were each dissolved in 5 ml of ether. One microliter of each ether solution was spotted on a planchet and counted for 1 min in an Atomic Instruments end-window proportional counter. The solvent was changed when the radioactivity of the sample dropped below 50 cpm/ μ l of ether solution.

Table 1 demonstrates that the recovery of both radioactivity and mass was quantitative. Individual fractions were combined as indicated in Table 1. The three major

TABLE 2 FATTY ACID COMPOSITION OF COLUMN PEAKS

Fatty Acid	Column Fractions		
	5-10	23-40	51-74
	% of total fraction		
14:0	0.7	—	—
15:0	0.6	—	—
16:0	56.6	—	—
16:1	—	1.4	—
17:0	0.7	—	—
18:0	38.5	—	—
18:1	—	98.0	0.9
18:2	—	—	99.0
20:0	1.0	—	—
20:1	—	0.7	—
22:0	0.8	—	—
24:0	1.0	—	—

Separations on F & M, model 500 Gas Chromatograph, using a 10 ft \times 1/4 inch i.d. stainless steel column packed with 15% EGSS-X on Gas Chrom P (support and stationary phase from Applied Science Laboratories, State College, Pa.). Carrier gas helium, with a flow rate of 55 ml/min at 190°. Quantitative results with National Heart Institute fatty acid ester standards showed that values between 1 and 5% have a maximum relative error of 10%; those above 5%, a maximum relative error of 5%.

peaks (fractions 5-10, 23-40, and 51-75) were assayed for radiochemical purity by reverse-phase paper chromatography (5) and for chemical purity by gas-liquid chromatography. The radiochemical data are shown in Fig. 2 and the GLC results in Table 2. These data demonstrate that the fatty acid methyl esters were satisfactorily separated according to their degree of unsaturation.

The separation reported above required a total of 15 hr. Similar columns have been used to separate the geometric isomers of octadecenoic and octadecadienoic acids.

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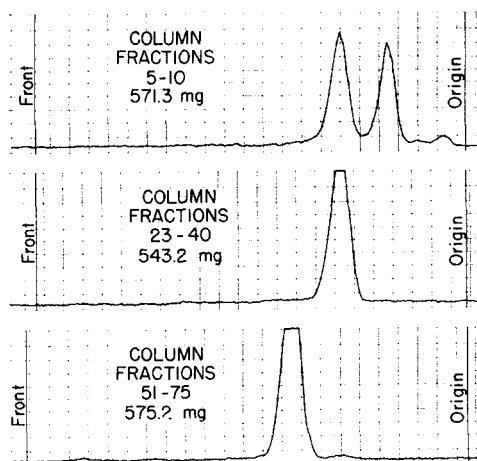


FIG. 2. Reverse-phase paper chromatographs of the three major peaks described in Table 1. Papers were scanned using an end-window G-M tube with a 1/2 inch slit-opening; scan speed 3 inches/hr. The total scale deflection is equal to 1,500 cpm. Major peaks correspond to stearate and palmitate (top); oleate (middle); and linoleate (bottom). See Table 2.