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## Separation of polyunsaturated fatty acid esters by argentation column chromatography on silicic acid

The ability of silver ions to form complexes with olefinic double bonds has been utilized<sup>1-5</sup> to separate fatty acids having different degrees of unsaturation. The present study describes the separation of fatty acid mixtures consisting of saturated and unsaturated esters up to hexaenoic acid.

Impregnation was carried out by mixing 100 g of silicic acid (100 mesh, Mallinckrodt, according to Ramsay and Patterson) with a solution of 20 g of silver nitrate in 250 ml of aqueous methanol containing 50 ml of water. The resulting slurry was dried to a free flowing powder in a rotary evaporator kept at about 100° by means of a water-bath. The dry powder was activated at 120° for about 16-18 h and stored in a dark bottle in a desiccator.

21 g of the coated silicic acid was mixed with 7 g of Celite, 80-120 mesh (B.D.H., for gas chromatography) and enough petroleum ether (b.p. 40-60°) to make a uniform slurry, most of which could be transferred in a single operation to a glass column (2 × 30 cm). The remaining slurry was then quickly transferred to the column by means of extra solvent. The column was allowed to settle to a final height of 17 cm and 5 g of powdered anhydrous sodium sulphate were placed on the top. The column was protected from light by wrapping it with black paper. After washing the column with 120 ml of petroleum ether (40-60°), a standard mixture was quantitatively transferred to it by an adequate volume of petroleum ether. The composition of the standard mixture of saturated and unsaturated (*cis*-isomers) fatty acid methyl esters is presented in Table I and was prepared by direct weighing.

The column was eluted batchwise with petroleum ether (40-60°) containing increasing amounts of diethyl ether at room temperature (*ca.* 24°). 10-ml fractions were collected by means of a fraction collector. The column was monitored by gas-liquid chromatography of each fraction after suitable reduction of the volume under

TABLE I

COMPOSITION OF STANDARD MIXTURES OF FATTY ACID METHYL ESTERS

Components	Weight in the mixture (mg)	Weight recovered (mg)	Eluent composition as % ether in petroleum ether (v/v)	Volume eluted (ml)	Tube nos. covered by the volume of eluent	Tube nos. containing components
C <sub>18:0</sub> <sup>a</sup>	18.8	18.2	2	200	1-20	10-19
C <sub>18:1</sub>	17.0	15.9	5	120	21-32	25-31
C <sub>18:2</sub>	19.0	17.5	10	120	33-44	36-43
C <sub>18:3</sub>	15.9	14.2	20	120	45-56	49-54
C <sub>20:4</sub>	21.2	20.1	35	120	57-68	58-66
C <sub>20:5</sub>	15.8	13.3	55	120	69-80	74-80
C <sub>22:6</sub>	15.0	12.2	90	120	81-92	85-91
Total	122.7	111.4		920		

<sup>a</sup> The first figure represents the chain length and the second figure the number of the double bond.

vacuum. The fractions under a particular peak were pooled, the solvent evaporated and weighed. They were then subjected to GLC analysis again to check on the possibility of overlapping components, if any. All the analyses were carried out by an F & M Model 700R Dual-column gas chromatograph with a flame ionization detector using a DEGS column on Gas-Chrom-Z (Applied Science Laboratories Inc., U.S.A.) at 170°. Nitrogen was used as the carrier gas.

Table I shows that there is good separation between any two adjacent classes of fatty acids. The losses of unsaturated esters, particularly polyenoates were appreciable. The details of the investigation along with its application to natural mixtures of fatty acid methyl esters will be published elsewhere.

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