

Use of Argentation Column Chromatography in the Identification of Fish Oil Fatty Acids by GLC: Application to Cod Liver Oil

ABSTRACT

The fatty acid methyl esters from cod liver oil have been fractionated into seven fractions, depending on degree of unsaturation, by a silver nitrate-treated silicic acid column. The fractions have been analyzed by gas liquid chromatography and the components identified by usual techniques.

The identification of fish oil fatty acids in a gas liquid chromatogram is a difficult problem, due to the presence of varieties of acids in these oils. Though the problem has been much simplified by Ackman and Burgher (1) with the development and use of the concept of separation factor, there remains the question of tentative identification. Silver nitrate-treated columns have been used by different workers (2,3) and more recently by the present authors (4) to separate a model mixture of fatty acids according to the degree of unsaturation. The present note reports the application of this fractionation technique to a natural sample, namely, cod liver oil. The mixed fatty acid methyl esters from the sample have been fractionated according to the degree of unsaturation prior to gas chromatography, whereby better accuracy in their tentative identifications has been achieved.

The fatty acids from cod liver oil were extracted by usual procedures using nitrogen atmosphere whenever required. Methyl esters of the mixed fatty acids were obtained by treatment with boron trifluoride-methanol

according to Metcalfe and Schmitz (5). A water-jacketed column (2 x 30 cm) containing silver nitrate (20%) coated silicic acid was used. The coating, column packing and elution technique were same as reported previously (4), where the column had been standardized with model mixture. A 100 mg portion of the methyl ester was applied on the column and eluted batch-wise with light petroleum ether (40-60) containing increasing amounts of diethyl ether. Each batch of eluent with definite composition was collected in an individual flask to comprise the respective fraction. The solvents in each of the flasks were evaporated under reduced pressure, the last traces being removed by a slow stream of nitrogen. The fractions were analyzed by a gas chromatogram (F & M Model 700-12R, Hewlett & Packard, Avondale, Pa.) using a 6 ft x 1/8 in. column containing 15% diethylene glycol succinate on 100-120 mesh Gas-Chrom P. The support and stationary phases were obtained from Applied Science Laboratories Inc., State College, Pa. Aliquots from each fraction were hydrogenated using platinum oxide catalyst in super dry methanol at room temperature and atmospheric pressure. Hydrogenated sample were analyzed under identical operational conditions. The component acids were identified using semilog plot, hydrogenation data, separation factors and previous reports by Ackman and coworkers (6,7). The results are presented in Table I.

There was good separation between any two adjacent bands. Trailing was observed in the cases of C_{18:1} and

TABLE I
Identification of Component Acids

Fraction no.	Degree of unsaturation	Eluent composition as % ether in petroleum ether, v/v	Volume eluted, ml	Component acids ^{a,b}
1.	Saturates	2	200	12-26 (even and odd), iso 12-20 (even and odd), anteiso 15,17, and 19
2.	Monoenes	5	120	14-24 (even and odd)
3.	Dienes	10	120	14,15,16(2),17,18(2),19,20(2),2122(2),23,24
4.	Trienes	20	120	16,17,18(2),19,20(2),21,22(2)
5.	Tetraenes	35	120	16,17,18,19,20(2),21,22,24(2)
6.	Pentaenes	55	120	20,21,22(2),24(2),26
7.	Hexaenes	90	120	22,24

^aThe figures represent the carbon chain length.

^bThe figures in parenthesis represent the number of isomers identified.

C_{20:5}, due to which traces of these components appeared in fractions 3 and 7, respectively. The trailing was due to the presence of large proportions of these acids in cod liver oil. The recovery of the esters of long chain highly unsaturated acids is probably not quantitative (3,4). The identity of the component acids was in good agreement with the reports by Ackman and coworkers. Details of the study will be published elsewhere.

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