Journal of Chromatography, 129 (1976) 469–470

CHROM. 9495

Note

Argentation column chromatography

A new ternary solvent system for the separation of methyl esters of polyunsaturated fatty acids

PARIMAL C. SEN, AMITABHA GHOSH and J. DUTTA
Department of Chemistry, Bose Institute, Calcutta 700009 (India)
(Received May 11th, 1976)

A method has been developed in this laboratory[1] for the separation of polyunsaturated fatty acids by argentation column chromatography according to the number of double bonds present up to hexaenoic acid. The charged column is eluted at 24° with light petroleum (b.p. 40–60°) containing increasing amounts of diethyl ether. Disadvantages of using this solvent system are: (a) the frequent need for restandardization, particularly in summer (30–35°), because of possible changes in the composition of the light petroleum; (b) the appreciable losses of components, particularly penta- and hexaenoate. The present note reports the results of the use of a ternary solvent system containing *n*-hexane with increasing amounts of ethyl methyl ketone and diethyl ether.

AR quality *n*-hexane was dried overnight on fused $CaCl_2$ and then distilled. The fraction boiling between 67 and 69° was collected. LR quality diethyl ether was freed from peroxides, washed with water, dried over fused $CaCl_2$, distilled and finally dried over metallic sodium wire. AR quality ethyl methyl ketone was distilled and the fraction boiling between 79 and 80° was collected. The preparation of the column

TABLE I

COMPOSITION OF THE STANDARD MIXTURE OF METHYL ESTERS OF FATTY ACIDS AND RECOVERIES OF THE COMPONENTS FROM THE COLUMN

Fatty acid methyl ester	*Weight in the mixture (mg)*	*Recovery (%)*	
		Present method	*Earlier method*[1]
18:0*	8.5	98.8	96.8
18:1	8.8	97.7	93.5
18:2	7.9	97.5	92.1
18:3	8.0	96.2	89.3
20:4	10.4	94.4	94.8
20:5	8.5	93.2	84.2
22:6	8.3	90.8	91.3

* The first figure represents the chain length and the second figure the number of the double bonds of the acid.

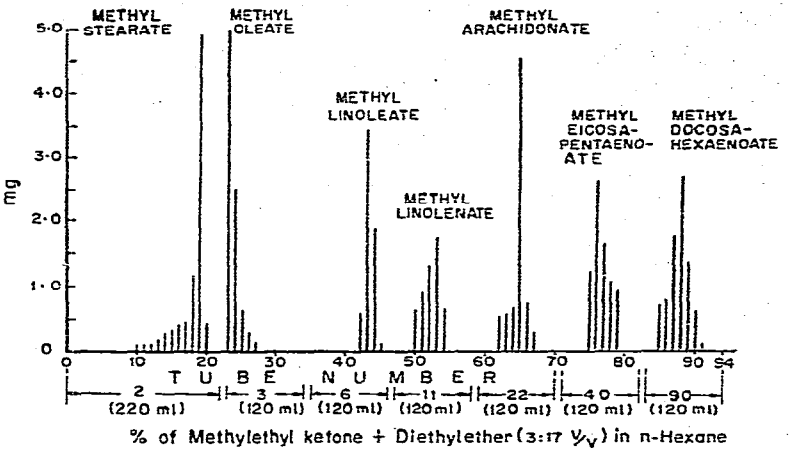

Fig. 1. Separation of standard fatty acid methyl esters (*ca.* 8 mg each) according to the number of double bonds present. The column (2 × 30 cm) was packed with a mixture of 21 g of 20% silver nitrate coated silicic acid (100 mesh, Mallinckrodt) and 7 g of Celite (80–120 mesh, BDH); temperature, 24 ± 0.5°. Fraction volume, 10 ml.

materials, packing and application of the sample were carried out as in the earlier method[1]. The sample was a standard mixture of methyl esters of saturated and unsatured (*cis*-isomers) fatty acids prepared by directly weighing components of 99% purity (Applied Science Labs., State College, Pa., U.S.A.). The composition of the mixture is shown in Table I.

The charged column was eluted batchwise at 24 ± 0.5° with *n*-hexane containing increasing amounts of a 3:17 mixture of ethyl methyl ketone and diethyl ether. 10-ml fractions were collected by means of a fraction collector. The amounts of the components in the collection tubes were estimated by direct weighing after removal of the solvent under nitrogen. In Fig. 1 the amount in each tube is shown against the number of the tube. The materials in each peak were pooled, diluted and analysed by gas–liquid chromatography (GLC) in order to check the possibility of overlapping of the components. The column and other conditions for GLC were the same as in the earlier method[1]. Better resolution (Fig. 1) as well as improved recovery of the components was obtained with the present solvent system (Table I). Reproducible results were obtained for one year provided the column temperature was kept at 24 ± 0.5°. The solvent system was successfully applied to the resolution of fatty acids of fish lipids. The results will be published elsewhere.

REFERENCE

1 A. Ghosh, M. Hoque and J. Dutta, *J. Chromatogr.*, 69 (1972) 207.