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Note

Separation of methyl esters of polyunsaturated fatty acids by argentation thin-layer chromatography

GAUTAM K. BANDYAPADHYAY and J. DUTTA*

Department of Biochemistry and Department of Chemistry, Bose Institute, Calcutta 700009 (India)

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Attempts have been made to separate esters of polyunsaturated fatty acids, both by thin-layer chromatography (TLC) and column chromatography, utilising the property of silver ions to form complexes with olefinic double bonds[1–11]. Silver ions enhance the polarity of the fatty acid esters to extents depending on the number of double bonds. This note describes the separation of a mixture of esters of fatty acids containing from 1 to 6 double bonds by argentation TLC, in readiness for their estimation by gas–liquid chromatography (GLC).

EXPERIMENTAL

A solution of the following fatty acid methyl esters (*cis*-isomers) was prepared in 2 ml of *n*-hexane: stearate ($C_{18:0}$); oleate ($C_{18:1}$); linoleate ($C_{18:2}$); linolenate ($C_{18:3}$); arachidonate ($C_{20:4}$); eicosapentaenoate ($C_{20:5}$) and docosahexanoate ($C_{22:6}$) (all obtained from The Hormel Institute, Austin, Minn., U.S.A.).

Thin-layer plates (14 × 28 cm) coated with a 0.5-mm layer of silica gel G (E. Merck, Darmstadt, G.F.R.) and impregnated with 7.5% silver nitrate were prepared from a slurry containing 0.53 g of silver nitrate, 15 ml of water and 7 g of silica gel. The plates were air-dried for 1 h and activated for 40 min at 110°.

The mixture of esters was applied in the form of a spot or band 1 cm from one of the shorter edges of a plate. A second (reference) spot of the mixture was also made on the line of application but at least 3–4 cm away from the first. The plate was then developed with three solvent systems. Solvent system 1, light petroleum (b.p. 40–60°)–diethyl ether–methanol–acetic acid (40:10:1:1) was applied up to 22 cm from the line of initial application. This was followed by development up to 26 cm with solvent system 2, light petroleum–diethyl ether–acetic acid (97:3:1). The portion of the plate containing the main spot was then covered with a glass plate while that containing the reference spot was sprayed with a 0.2% ethanolic solution of 2′,7′-dichlorofluorescein. The position of the ester having 3 double bonds ($C_{18:3}$) was noted under UV light. Final development with solvent system 3, light petroleum–diethyl ether–acetone–acetic acid (10:40:3:1), was continued until the solvent front had migrated

* To whom reprint requests should be sent.

to *ca.* 3–4 mm below the position of the $C_{18:3}$ ester. The length of the final development was *ca.* 10–11 cm from the line of application. Solvents were removed from the plate under vacuum after each development. The total time for all three developments was *ca.* 3 h. Room temperature was maintained at 25°. The plate was always sprayed very lightly in order to keep contamination of the esters by the dye to a minimum in the subsequent estimation steps.

For estimation, the mixture of esters was applied as a band. After separation, bands due to the individual esters were scraped off carefully along the margin. Material from each band was placed in a 12-ml centrifuge tube and 5 ml of acetone–diethyl ether (9:1) was added. The tube was capped, kept for 1 h with frequent stirring and then centrifuged. The resulting supernatant was then set aside. The extraction procedure was repeated three times with the residue. The pooled supernatants were concentrated under vacuum and reduced to a volume suitable for analysis. A known

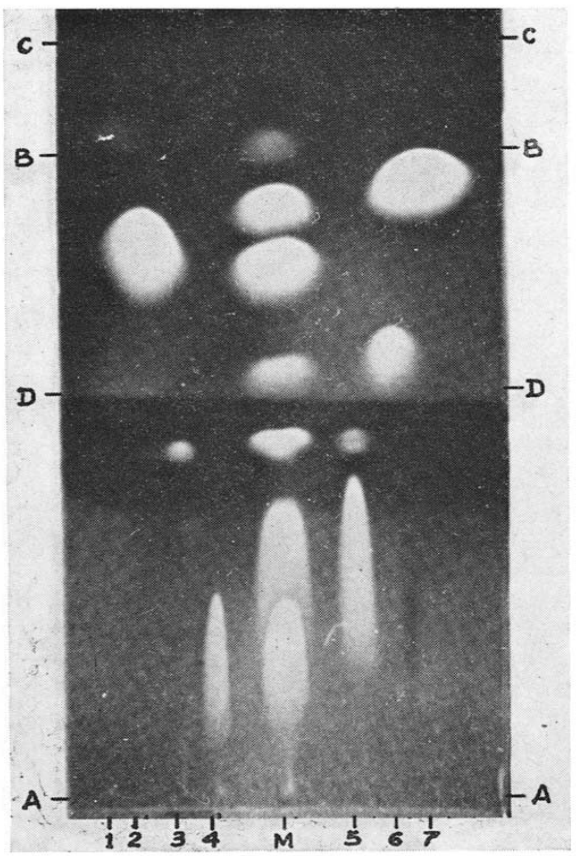

Fig. 1. Argentation thin-layer chromatogram of methyl esters of fatty acids containing 0–6 double bonds. A = Line of application; A–B = development with solvent system 1; A–C = development with solvent system 2; A–D = development with solvent system 3. 1 = Stearate; 2 = linoleate; 3 = arachidonate; 4 = 4, 7, 10, 13, 16, 19-docosahexaenoate; 5 = 5, 8, 11, 14, 17-eicosapentaenoate; 6 = linolenate; 7 = oleate; M = mixture of 1 to 7. The photograph was taken under UV light after spraying the chromatogram. The reference spot was not used.

volume of a standard solution of either methyl pentadecanoate ($C_{15:0}$) or methyl nanodecanoate ($C_{19:0}$) was then added. The former standard was used for $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ esters, and the latter for $C_{20:4}$, $C_{20:5}$ and $C_{22:6}$. These mixtures were subjected to GLC analysis using a column of 10% DEGS on Gas-Chrom Z. The amount of each fatty acid ester was determined from the chromatogram using the relation:

$$\frac{\text{Amount of fatty acid ester}}{\text{Amount of standard fatty acid ester added}} = \frac{\text{Area of peak due to fatty acid ester}}{\text{Area of peak due to the standard ester}}$$

Fig. 1 shows the qualitative separation of fatty acid esters on a TLC plate. According to the GLC analysis, the band was not contaminated with other components. More than 90% of the applied material was recovered. Extraction of the material involves breaking of complexes formed between the silver ions and the unsaturated fatty acids. This process is probably time dependent and the time factor becomes progressively more stringent with increase in the degree of unsaturation. We used a high-polarity organic solvent system and allowed sufficient time for extraction, otherwise the fatty acids with higher degrees of unsaturation would have suffered appreciable loss. The above technique is of use for the quantitative isolation and qualitative study of polyunsaturated fatty acids from natural sources of lipids.

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