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Notes on Methodology

A simple method for the separation of minute amounts of tissue lipids by thin-layer chromatography and gas-liquid chromatography

MILADA DOBIÁŠOVÁ

Isotope Laboratory,
Institute for Biological Research,
Czechoslovak Academy of Sciences,
Czechoslovakia, Prague 6

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» In a study of the content of higher fatty acids in different lipid fractions of rat organs during postnatal development, it was necessary to adapt some of the known techniques, particularly the combined use of thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) (1–5). A technique has been devised that makes it possible to treat a large number of samples within a relatively short period of time.

Lipids of lungs, livers, intestine, and white and brown adipose tissue were extracted with Bloor's solution, ethanol–diethyl ether 3:1 (v/v). The extracts were dried in vacuo, and aliquots of 2–15 mg were transferred to 2-ml glass ampoules in a small amount of chloroform–methanol 2:1.

Analytical thin-layer chromatography was performed according to Peifer (6) on microscope slides (2.5 x 8 cm) using Silica Gel G[1]. The chromatograms were developed in hexane–diethyl ether–ethyl acetate 40:10:1.5 (v/v) and detected by charring after having been sprayed with 25% perchloric acid in water. Phospholipids, cholesterol, free fatty acids, triglycerides, and cholesterol esters were readily distinguished by this procedure. Diglycerides appear at the same spot as cholesterol.

Preparative thin-layer chromatography of phospholipids, triglycerides, cholesterol, and cholesterol esters was performed on glass plates (8 x 20 cm) covered with silica gel (7) (200–400 mesh), the layer being 0.5 mm thick. Silica gel was saturated with water to 10%. Samples (2–15 mg) were applied in a line 2 cm from the bottom edge of the plate. Chromatograms were developed with hexane–diethyl ether–ethyl acetate 40:10:1.5 (v/v) for 15–18 min. After evaporation of the solvent, the individual fractions were identified. About 1 cm of the edge of another plate of the same dimensions was coated with silicic acid; this was pressed on the marginal strip of the preparative chromatogram and a print was taken. The print was then detected by the usual technique. A similar principle has been used by Reichelt and Pitra (8) for the identification of steroids. The identified regions (R_F values: phospholipids 0.00, cholesterol 0.16, triglycerides 0.67, cholesterol esters 0.85) were then sucked into conical glass tubes 6 mm in diameter. The lipids were extracted from the silica gel with chloroform–methanol 2:1 and placed in 2-ml ampoules. At least 85% of the lipids was thus eluted. All the ampoules were placed into a vacuum desiccator, and the solvent was evaporated at 37° in vacuo. The purity of the fractions was again controlled on microscope slides.

Methanolysis of the various lipid classes was carried out in the same ampoules according to the procedure of Stoffel, Chu, and Ahrens (4). One drop of benzene and 0.3–0.4 ml 5% HCl in absolute methanol were added. The ampoules were sealed and heated at 70° in a thermostatically controlled oven for 3 hr. After cooling to room temperature, the ampoules were opened and an equal volume of distilled water was added. The methyl esters were extracted three times with 0.3 ml petroleum ether, bp 25–50°. The petroleum ether layer was collected into capillary tubes and transferred to clean ampoules. The solvent was evaporated to dryness, and ampoules were sealed and kept at −10° for GLC. A Pye argon chromatograph was employed.

The remainder of the sample in the ampoule was hydrogenated in 0.3 ml absolute methanol with 1 mg of Adam's platinum dioxide catalyst under slight hydrogen pressure for 30 min. The ampoules were kept under hydrogen until the next day. The solution was then transferred to another ampoule, the solvent was evaporated, and the sample was ready for GLC. The yields were quantitative.

In the case of the cholesterol ester fraction and the mixed fraction of cholesterol and diglyceride, small columns of silica gel (7) (150–300 mesh, column diameter 0.4 cm, length 5 cm), washed with methanol, acetone, diethyl ether, and pentane (9), were used for the separation of cholesterol from the methyl esters of fatty acids. Samples up to 2 mg were applied in a small volume of pentane. The methyl esters of fatty acids were eluted with 2 ml 10% diethyl ether in pentane. The sterols were extracted into chloroform–methanol.

The above method is relatively rapid and effective when analyzing large numbers of samples that contain small amounts of lipids.

[1] Silica Gel G for thin-layer chromatography according to E. Stahl was obtained from E. Merck A. G., Darmstadt, Germany.

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