Journal of Chromatography, 86 (1973) 197–199

CHROM. 6901

Note

Two-step, two-dimensional development thin-layer chromatography of lipids on a micro-scale

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(Received June 25th, 1973)

Lipids can be fractionated by thin-layer chromatography in a number of ways[1,2]. This paper describes a two-step, two-directional development thin-layer chromatographic procedure using three solvent systems for the resolution of complex mixtures of lipids, *e.g.*, serum lipids. Thin-layer chromatography of lipids on a micro-scale has a number of advantages when compared with the macro-scale method. It is possible, for example, to separate as little as 10^{-9} g of a lipid extract, which enables lipid patterns at the cellular level to be studied. Furthermore, the micro-scale procedure described can be completed much more rapidly than the macro-scale method. The whole operation takes less than 1 h, whereas conventional lipid thin-layer chromatography can take about 6 h, including chamber saturation. In addition, the quality of the separation is much better, owing to the small distance required for development, which minimizes the diffusion of the lipid into the surrounding areas. Moreover, solvent systems that migrate slowly can be used to separate lipids on a micro-scale, while such solvents are inapplicable in normal procedures.

EXPERIMENTAL AND RESULTS

An object slide was cut to 45×26 mm, carefully cleaned with chromic acid and then rinsed with copious water and chloroform–methanol. The micro-plate is held at one corner with a pair of forceps and dipped into a slurry of silica gel (silica gel Camag D-O; Camag, Muttenz, Switzerland) in chloroform and dried in a horizontal position. Excess of silica gel on the back of the plate is wiped off, and the thin-layer chromatographic micro-plate is pre-cleaned by chromatography with a solvent that is a combination of the solvent systems to be described. After drying, the plate is ready for use. A lipid extract in chloroform–methanol (2:1) is applied to one corner of the plate under stereovision. The applied spot (diameter 1 mm) should be small and an appropriate size is achieved by use of a self-drawn glass capillary. A distance of 1.5 cm below the upper margin is clearly marked. The spot of the lipid is then developed with solvent system A (chloroform–methanol–twice-distilled water, 65:25:4), and the chromatography is terminated when the solvent

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front has reached the marking. After drying the plate, a second development with solvent system B (*n*-hexane–diethyl ether–glacial acetic acid, 85:20:2) in the same direction is carried out, and the solvent front is allowed to reach the top edge of the plate. The chromatogram is then turned through 90°, and a third development is allowed to proceed using solvent system B. In order to isolate one part of the plate so that the other can still be used for chromatography, silica gel is removed with a spatula as shown in Fig. 1. Solvent system C (*n*-butanol–glacial acetic acid–twice-distilled water, 60:20:20) is then used to resolve the lower part of the plate only, by development in the second direction up to the edge of the plate. Chromatography is carried out in covered beakers. Before placing the plates into the beakers, a small amount of the solvent system (0.4–0.5 ml of solvent for 50 and 150-ml beakers) is introduced. The first development requires the beaker to be coated with a solvent-saturated filter-paper. The lipids are rendered visible as yellow spots in an iodine atmosphere. The scheme shown in Fig. 1 outlines the experimental procedure.

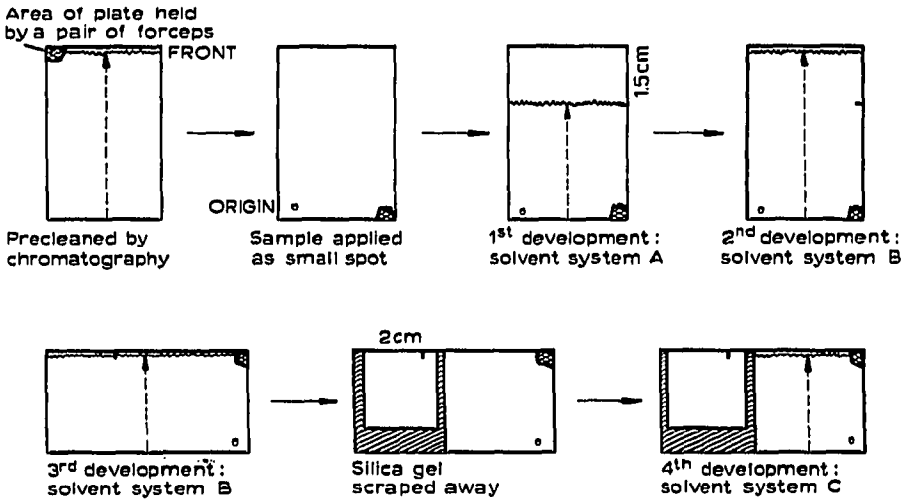

Fig. 1. Scheme of the two-step, two-directional development thin-layer chromatography of lipids on a micro-scale.

Human serum lipids give the chromatographic pattern shown in Fig. 2. The serum lipids were prepared as follows. Blood was drawn from donors and centrifuged, and the sera from a number of samples were separated and pooled. After freeze-drying of the pooled serum, a crude lipid extract was made by extraction with an excess of chloroform–methanol (2:1), and evaporated to dryness. From this preparation, a pure lipid sample was extracted by use of chloroform–diethyl ether (1:1). The same extraction procedure can be used to isolate tissue or single-cell lipids after homogenization[3,4].

The subsequent quantitative determination of these exceedingly small amounts of lipids on the chromatogram cannot be carried out by classical methods, because the concentrations are far below the limit of detection. The spots of the lipids are scraped off the micro-plate and extracted from the silica gel with chloroform–methanol (2:1), as reported for phospholipids, and thereafter analyzed fluorimetrically

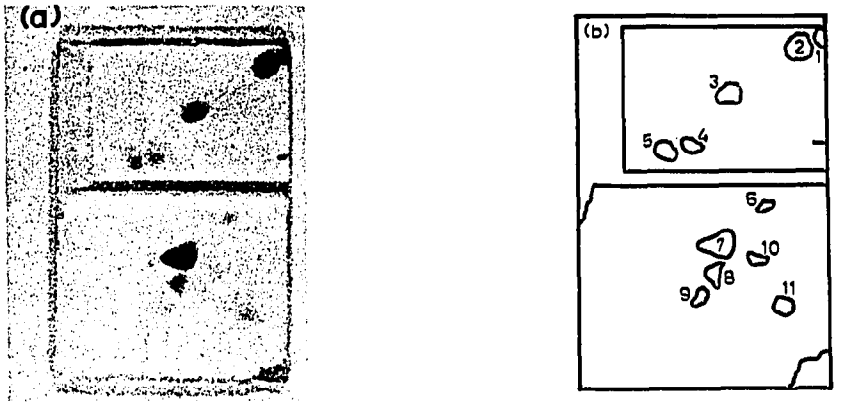

Fig. 2. Two-step, two-directional development thin-layer chromatogram of serum lipids on a micro-scale (a) and a "map" (b) showing the localization of the spots. 1, Front; 2, cholesterol esters; 3, triglycerides; 4, free fatty acids; 5, cholesterol; 6, cephalin; 7, lecithin; 8, sphingomyelin; 9, lysolecithin; 10 and 11, not identified.

with rhodamine in a spectrofluorimeter[5]. However, the most efficient method is direct quantitative photometry on thin-layer chromatograms[6]. The spots are measured on the plate by absorption photometry in remission or by fluorimetry, and in this so-called "*in situ* measurement", sensitivity at the nanogram level is achieved.

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