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Letter to the Editor

Improved one-dimensional thin-layer chromatography for the separation of phospholipids in biological material

Sir,

In 1981, Jensen and Hedegaard [1] reported a one-dimensional horizontal thin-layer chromatographic (TLC) technique for phospholipid separation using chloroform–methanol–2-propanol–0.25% KCl–ethyl acetate (30:9:25:6:18) as the solvent system. The following lipids in a sample of brain lipids were separated: sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, phosphatidylethanolamine and cerebrosides.

The aims of our study were to use a simpler multiple ascending technique instead of horizontal development and to extend the range of the substances to be separated.

EXPERIMENTAL

Materials

Phospholipid standards and other reference samples were purchased from Koch-Light (Colnbrook, U.K.) or isolated in our laboratory Tissue samples were extracted twice with chloroform–methanol (2:1, v/v) and the amount of the extract corresponding to 50–100 n*M* lipid phosphorus was spotted on the 0.5-cm startline

Chromatography

High-performance thin-layer chromatographic (HPTLC) plates silica gel 60 (Merck, Darmstadt, F.R.G.), 10 × 10 cm, were used. Chromatography was carried out using the solvent mixture chloroform–methanol–2-propanol–0.25% KCl–ethyl acetate (30:9:25:6.18) in a saturated chamber (12 × 12 × 5 cm) at laboratory temperature (20 ± 1°C).

For good separation, it was necessary to repeat the development four or five times. Detection was made with Vaskovsky's [2] and orcinol reagents [3].

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RESULTS AND DISCUSSION

A comparison between the original method and our modification is given in Fig. 1.

With the aid of standard phospholipids supplied by the Koch-Light company, and other standard reference samples prepared in our laboratory, we were able to determine as extra values compared with the original report [1] the mobility of lysophosphatidylcholine, lysophosphatidylethanolamine, diphosphatidylglycerol and bis(monoacylglyceryl)phosphate (BMP) As for glycolipids we were able to determine for the first time the mobility of ceramide monohexosides, ceramide dihexosides, ceramide trihexosides, ceramide tetrahexosides and sulphatides.

Using the above-mentioned chromatographic procedure, it is difficult to distinguish phosphatidic acid from diphosphatidylglycercol. Their mobilities are only slightly different. All other phospholipids are well separated.

Neutral lipids, cholesterol and chlolesteryl esters move near or with solvent front.

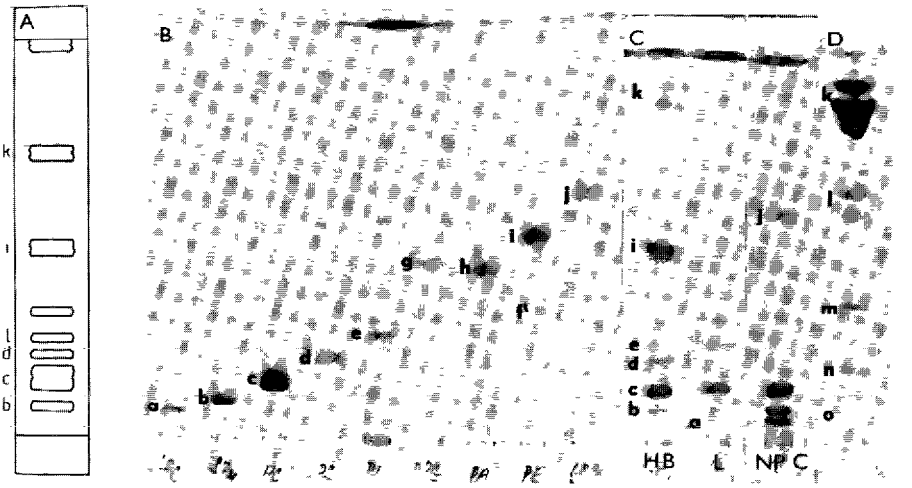

Fig. 1. (A) Separation achieved by original method [1], using two-fold horizontal development in chloroform–methanol–2-propanol–0 25% aqueous KCl–ethyl acetate (30:9 25 6 18) (B–D) Our modification using four-fold ascending development in the same solvent mixture: (B) reference standards of phospholipids, (C) lipid extracts from human brain (HB), liver (L) and brain of Niemann–Pick type C (NPC), samples L and NPC were briefly formol fixed, (D) mixture of glycolipids Detection: B and C, iodine vapour, D, orcinol–H_2SO_4 reagent Phospholipids: a = lysophosphatidylcholine; b = sphingomyelin; c = phosphatidylcholine, d = phosphatidylserine, e = phosphatidylinositol; f = lysophosphatidylethanolamine, g = diphosphatidylglycerol; h = phosphatidic acid, i = phosphatidylethanolamine, j = bis-(monoacylglyceryl) phosphate Glycolipids k = ceramide monohexosides; l = ceramide dihexosides, m = sulphatides, n = ceramide trihexosides, o = ceramide tetrahexosides Sample quantitation lipid standards, 4–40 μg per start, tissue extracts, 50–100 nM lipid phosphorus per start

Our modification permits the separation of more phospholipid fractions as the methods described earlier [1,4]. Although two-dimensional chromatography enables detailed separation, the multiple one-dimensional technique has the advantage of plate savings. We recommend four-fold development in humid weather and five-fold development in a dry atmosphere.

The problem of overlapping of some phospholipid and glycolipid zones can be solved by different detection methods, *e.g.* lipid phosphorus [2] or orcinol reactions [3]. For the quantitative determination of phospholipids the photometric method according to Rouser and co-workers [5,6] was used

In our laboratory, we use this technique of separation to study phospholipids in Niemann–Pick disease, where it is necessary to monitor not only sphingomyelin, stored extensively in types A and B, but also minor fractions such as BMP and acylphosphatidylglycerol. The mobility of acylphosphatidylglycerol is between those of BMP and neutral lipids. This lipid is rarely detectable in traces in samples from Niemann–Pick patients. In type C of the disease, the increase in sphingomyelin concentration is not quite so prominent, though it does make diagnostic sense to study it just as much as the other minor fractions.

A comparison between pathological and control samples in a single chromatogram permits changes in the minor fractions to be observed directly. For clear detection even of the minor fractions it is sufficient to apply an extract corresponding to 4 mg of hepatic tissue at the 0.5-cm startline, *i.e.* an amount obtainable from bioptical samples.

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