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Note

Simple preparative and analytical thin-layer chromatographic method for the rapid isolation of phosphatidic acid from tissue lipid extracts*

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The accurate assessment of phosphatidic acid in biological samples is very important, since this compound is a key intermediate in lipid biosynthesis. In addition, certain phosphatidic acid pools are involved, through phosphatidate phosphatase, diglyceride kinase and CDPdiglyceride synthetase, in the "neurotransmitter effect" exerted in the nervous tissue[1]. Moreover, because of the metabolic heterogeneity of phosphatidic acid[2–4], the separation of the molecular species involved and the determination of their fatty acid compositions is of interest.

The available techniques for the preparative isolation of phosphatidic acid from tissue lipid extracts are relatively time-consuming, involving column and then thin-layer chromatography (TLC)[2–5], or alkaline alcoholysis of washed total-lipid extracts followed by isolation of the glycerophosphate by electrophoresis[6,7] or two-dimensional paper chromatography[8]; the last procedure also converts any lysophosphatidic acid in the tissue into glycerophosphate. A rapid analytical procedure useful for the isolation of phosphatidic acid is two-dimensional TLC[9,10], which, although it satisfactorily resolves several phospholipids, implies the use of one plate per sample. Thus, there is need for a rapid procedure for the quantitative isolation of phosphatidic acid on a preparative scale and that will also permit analytical separation of several samples simultaneously. We describe here an oblique-spotting technique used with two-dimensional TLC, which fulfils these requirements.

Extraction of the tissue lipids should be made essentially as previously described[11], with the addition of washings of extracts maintained 0.04 *M* in calcium chloride to avoid loss of phosphatidic acid[6]. Silica gel H layers prepared with 3% of magnesium acetate should be used[6], but the layer thickness may be varied according to the phosphatidic acid concentration in the samples and the amount of total lipids.

A Plexiglas cover[12] modified as follows can conveniently be used to apply the spots obliquely in an atmosphere of nitrogen. The syringe needle or micropipette is introduced through an oblique slit (3 mm wide × 100 mm long) in the top of the Plexiglas cover at an angle of 45° from one corner. Fig. 1 shows the chromatographic

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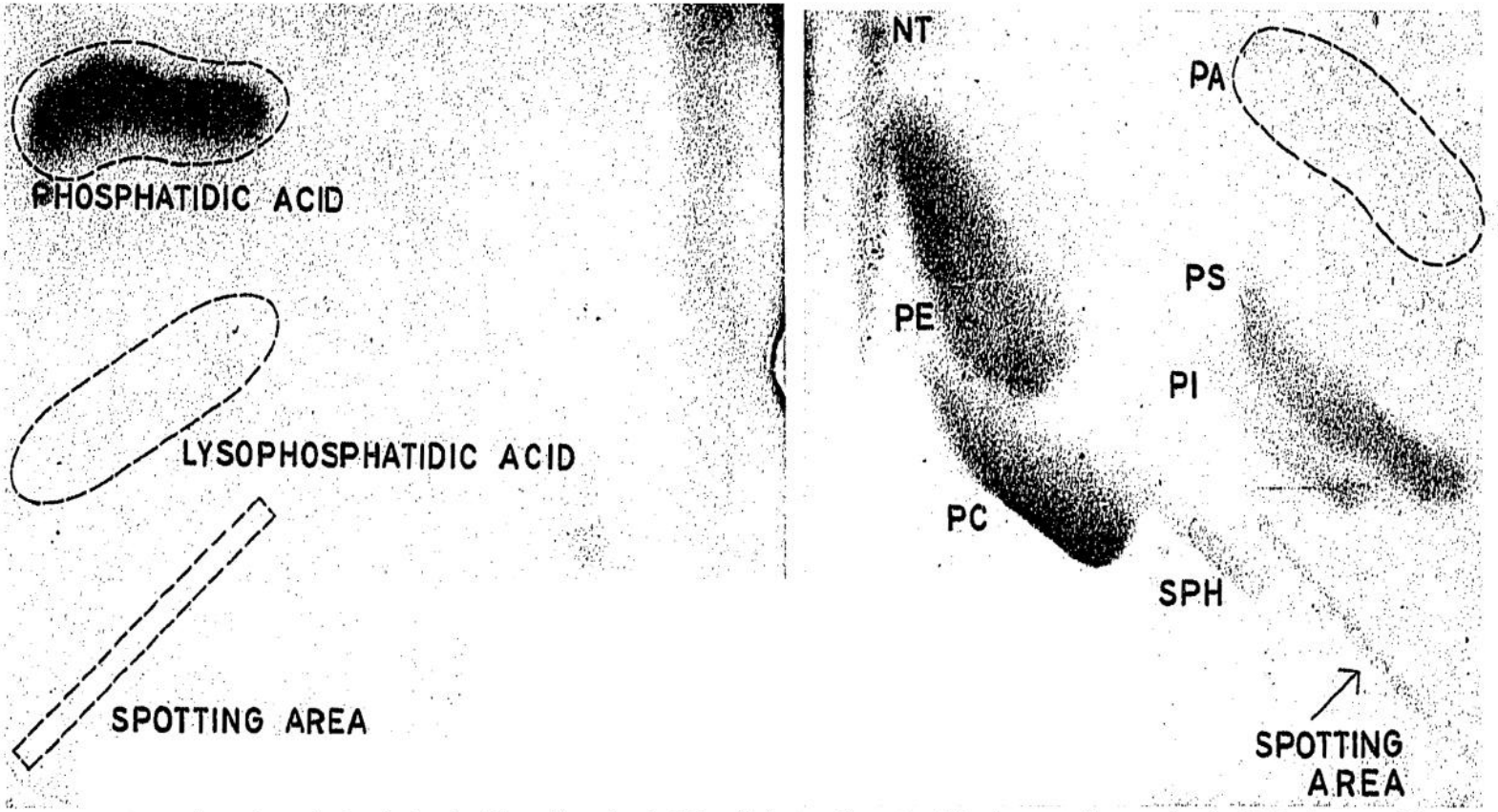

Fig. 1. Separation of standards of phosphatidic and lysophosphatidic acids by two-dimensional development after spotting the lipids along an oblique origin; the spotting area may be located in different positions, so that the directions of development may be as shown here or as in Figs. 2 and 3.

Fig. 2. Preparative two-dimensional TLC with oblique spotting for isolation of phosphatidic acid (PA) from an extract of mouse-brain lipids; the dashed circle indicates the area containing PA. Visualization was made by charring with a copper acetate reagent[13]. The direction of development can be modified, as here, in comparison to Fig. 1, without affecting separation. NT = neutral lipid; PE = phosphatidylethanolamine; PC = phosphatidylcholine; PS = phosphatidylserine; PI = phosphatidylinositol; SPH = sphingomyelin.

behaviour of a phosphatidic acid that contained a minor amount of lysophosphatidic acid. Thus, the sample may be applied as an oblique streak or as several round spots along the line of the slit. By applying streaks, one or two samples per plate may be applied for isolating the phosphatidic acid on the preparative scale (Fig. 2); by applying spots, six or seven samples may be run per plate, so obtaining analytical separations (Fig. 3). The depicted direction of oblique spotting prevents other lipids from over-running the phosphatidic acid during development. In both instances, two-dimensional development is carried out, with chloroform–methanol–ammonia solution (65:25:15) and subsequently chloroform–acetone–methanol–acetic acid–water (6:8:2:2:1) as mobile phase, as described by Rouser *et al.*[9]; it is advisable to overrun for approx. 1 h with the former solvent system. When further analyses are intended, such as isolation of molecular species, care must be taken to avoid formation of peroxides; thus, after development in each direction, the surface of the layer should be flushed with dry nitrogen to accelerate evaporation. This can conveniently be done by connecting the nitrogen supply to a plastic box (3 × 5 × 0.5 cm) having several small holes along one of its edges to spread the gas stream. The chromatograms so obtained permit isolation of the phosphatidic acid, whereas other phospholipids are completely or partially overlapped (Figs. 2 and 3).

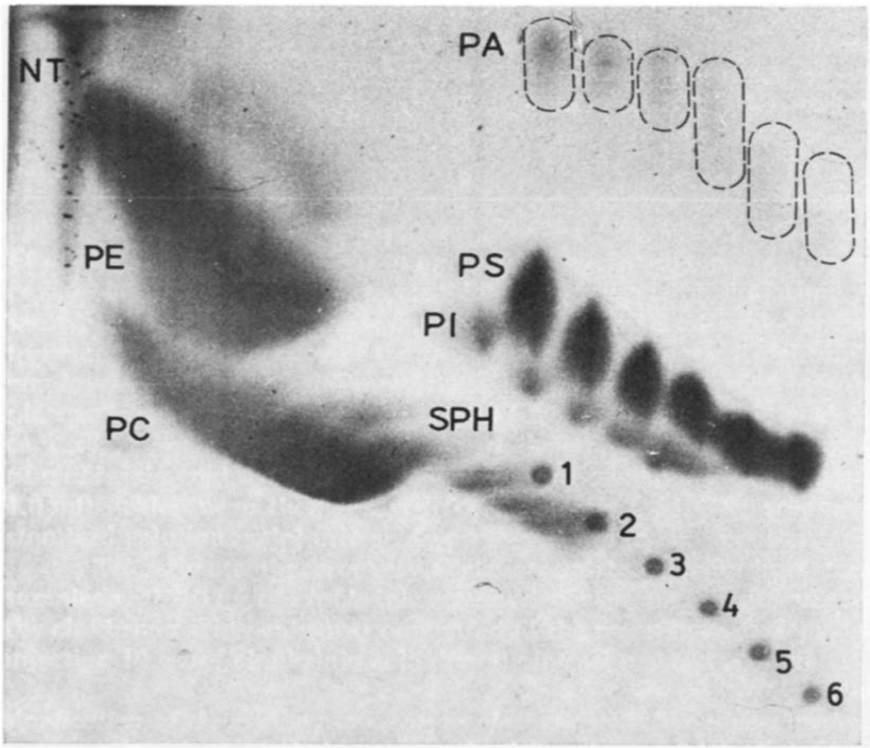

Fig. 3. Analytical two-dimensional TLC after spotting circular areas (1–6) along an oblique line. The dash-encircled areas show phosphatidic acid (PA) separated from mouse-brain-lipid extracts; abbreviations are as in Fig. 2. The direction of development can be modified, as here, in comparison to Fig. 1, without affecting separation.

If it is desired to separate other phospholipid classes, glass plates (40 × 20 cm) can be used. Thus, by suitable choice of development direction and plate size, phosphatidic acid as well as other phosphoglycerides may be isolated on a preparative or analytical scale from complex tissue-lipid extracts by oblique spotting.

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