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Simplified preparative thin-layer chromatography of phospholipids

Thin-layer chromatography has proven to be a simple technique for separation of a mixture of phospholipids since one can achieve a separation in a short time[1–4]. However, a complete separation of the complex mixture of phospholipids from platelets is not attainable on a single plate. For the purpose of isolating a sufficient quantity of pure phospholipids for fatty acid analyses, we have found it necessary to rechromatograph individual bands containing a mixture of phospholipids. In order to eliminate many of the time consuming operations of rechromatography, we have evolved a modification of the basic procedure of preparative TLC.

In carrying out the modification, the usual steps for unidimensional thin-layer chromatography are followed. A lipid extract is applied as a streak along the origin of a plate (Silica Gel HR), developed with chloroform–methanol–water (65:30:5, by vol)[3]. Fig. 1 shows the separation of the major phospholipids after the first separation. The bands containing a mixture of phospholipids are viewed under an ultraviolet lamp after spraying the plate with dichlorofluorescein and the spots are outlined with a pointed instrument. The outlined areas are removed from the plate by scraping with a spatula into a beaker or test tube. A slurry is made with chloroform–methanol (5:1, by vol.) with the silica gel in a ratio of 1:1, by vol. The slurry is applied from a capillary pipette or syringe along the origin of a second plate (see Fig. 2). The application of the slurry should be such that it forms a smooth, low mound, taking care that no sharp peaks are formed on the surface. The solvent is evaporated from the mound

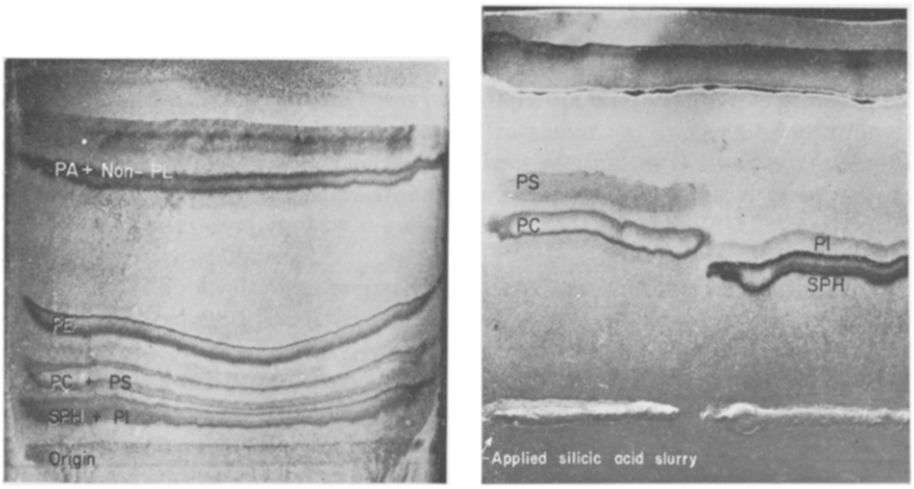

Fig. 1. Thin-layer chromatogram of a lipid extract of human platelets. SPH + PI = sphingomyelin plus phosphatidyl inositol; PC + PS = phosphatidyl choline plus phosphatidyl serine; PE = phosphatidyl ethanolamine; PA + non-PL = phosphatidic acid plus non-phospholipids. Thin layer: Silica Gel HR. Solvent: chloroform–methanol–water (65:30:5).

Fig. 2. Thin-layer chromatogram of bands SPH + PI and PC + PS taken from the thin-layer plate shown in Fig. 1 and rechromatographed. Thin layer: Silica Gel HR. Solvent: chloroform–methanol–water–HAc (80:40:5:7).

by applying a gentle stream of nitrogen over the surface. When the bottom surface of the glass above the mound returns to ambient temperature, the plate is carefully placed in a tank containing the second solvent system (chloroform–methanol–acetic acid–water, 80:40:5:7, by vol.)[5]. Fig. 2 shows the separation of the mixture of phosphatidyl choline and phosphatidyl serine into two discreet bands and a similar separation is shown for phosphatidyl inositol and sphingomyelin. Separations have been achieved with mixtures of known standards and extracts of lipids from platelets and erythrocytes, and result in essentially the same separation as is obtained by using the longer conventional procedure.

The simplified procedure has been utilized in our laboratory for the analysis of fatty acids of phospholipids by GLC. We have found that this procedure is much less laborious than the original procedure, saving several hours during each run. The length of the time saved can be substantial when a series of samples are processed.

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