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# A method for the one-dimensional thin-layer chromatographic separation of serum phospholipids*

The requirement for a rapid analytical procedure for the resolution and quantitation of the principal serum phospholipid classes, utilizing TLC separations, led to the development of this method. To meet the requirements of the desired analytical procedure, the method had to exhibit the following characteristics: one-dimensional separation to accommodate multiple samples per plate, resolution of the principal phospholipid classes with minimal overlapping, stable and reproducible operation on a day-to-day basis, and adaptability to densitometric scanning for quantitation. Attempts to use reported systems did not permit precise resolution of individual lipid classes in one dimension[1–4], and therefore the following method was developed.

*Materials and methods*

Glass plates and spreader were obtained from Quickfit Reeve Angel, Clifton, N.J., U.S.A. Adsorbosil-5 silica gel was obtained from Applied Science Laboratories, State College, Pa., U.S.A. This silica gel was selected after testing of numerous other commercially available silica gels. Spectroquality chloroform and methanol, and chromatoquality tetrahydrofuran were obtained from Matheson, Coleman and Bell, East Rutherford, N.J., U.S.A. The individual lipid standards and mixture were obtained from Supelco, Inc., Bellefonte, Pa., U.S.A. Spotting syringes and dispenser were obtained from Hamilton Company, Whittier, Calif., U.S.A.

The 20 × 20 cm glass plates were coated with a 250 $\mu$ thick slurry of silica gel and allowed to air-dry at ambient temperature for about 2 h. The air-dried plates were scribed into 1.0 cm wide lanes and activated in a convection oven at 110° for 20 min. After activation, the plates were cooled in a nitrogen-flushed desiccator containing silica gel desiccant. Routinely a sample was spotted under nitrogen in a narrow band in the center third of a lane. Each sample contained 100 $\mu$g of lipid in 10 $\mu$l of chloroform–methanol (95:5). After evaporation of the spotting solvent, the plate was developed in a paper-lined TLC tank for 9.0 min in tetrahydrofuran–methanol (3:1). The plate was allowed to dry for 20 min under dry nitrogen and then transferred to a second paper-lined TLC tank and developed in the same dimension to a height of 17 cm in chloroform–methanol–4 *M* ammonium hydroxide (75:37:7). The plate was then removed from the tank, dried at ambient temperature, sprayed with a 20% aqueous solution of ammonium bisulfate[5] and charred 90 min at 170° for detection.

*Discussion*

Fig. 1 illustrates a typical chromatogram obtained by the above method with phospholipid standards. Fig. 2 illustrates a typical chromatogram showing the separation of phospholipid classes obtained by this method with a phospholipid fraction isolated from human serum. Changes in room temperature, relative humidity (10–60%)

* The following abbreviations will be used in this manuscript: LPC = lysophosphatidylcholine, SPH = sphingomyelin, PC = phosphatidylcholine, PS = phosphatidylserine; PI = phosphatidylinositol; PE = phosphatidylethanolamine, PG = phosphatidylglycerol, CAR = cardiolipin; PA = phosphatidic acid, MIX = mixture of all nine phospholipids listed above.

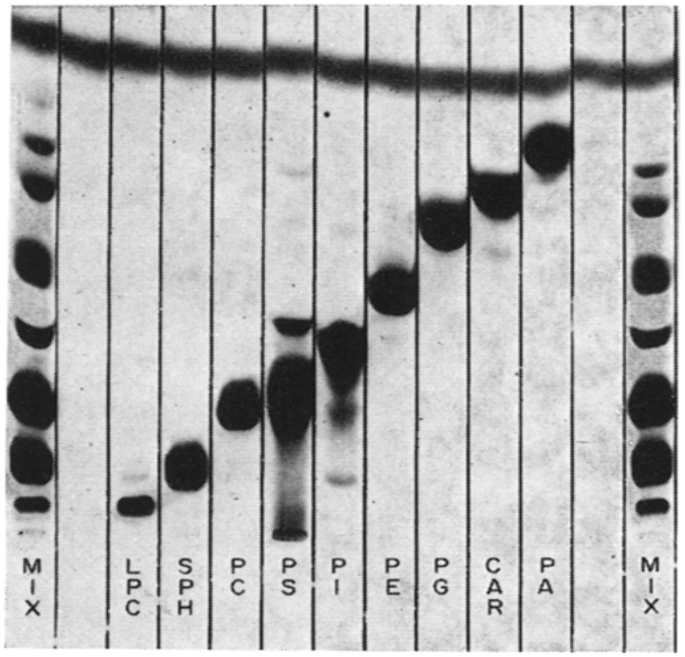


Fig. 1. Thin-layer chromatogram of standard phospholipid classes.

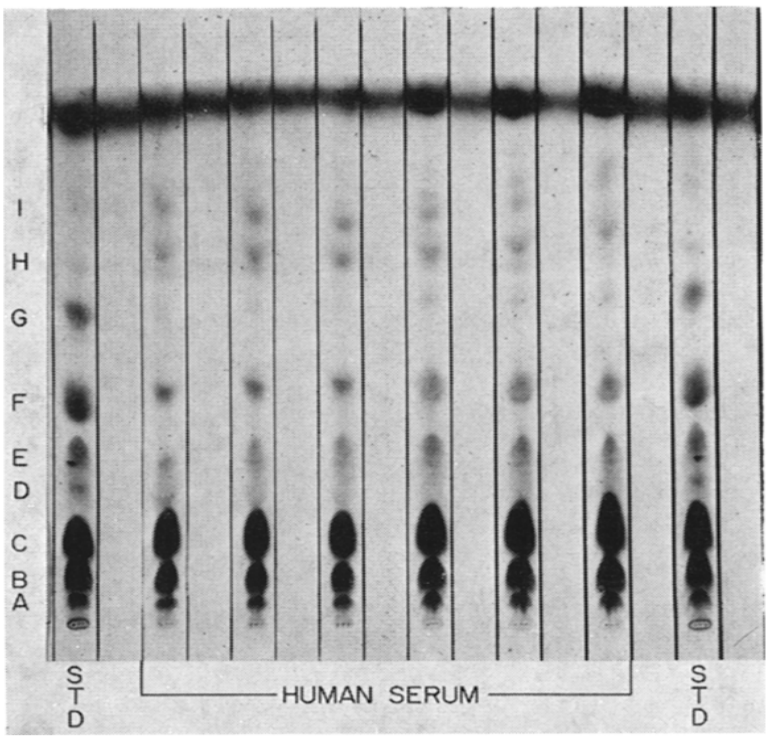


Fig. 2. Thin-layer chromatogram of human serum phospholipids (six inside lanes) and a standard phospholipid mixture (two outside lanes). A = Lysophosphatidylcholine, B = sphingomyelin, C = phosphatidylcholine; D = phosphatidylserine, E = phosphatidylinositol, F = phosphatidylethanolamine; G = phosphatidylglycerol; H = cardiolipin and I = phosphatidic acid.

and lot variations in silica gel result in changes in the observed separations. These changes can be corrected by varying the volumes of methanol and ammonium hydroxide solution used in the second developing solvent. Separations between PG and CAR vary slightly with the activation time. Under some conditions, this pair will not separate on plates activated for longer or shorter times. The separation of PS is somewhat variable, and the conditions controlling this separation are not completely understood at this time. In our experience tetrahydrofuran has proven to be unstable during storage. Placing an iron nail or paper clip into the bottle when first opened, flushing with nitrogen, and storage at 4° enable the compound to be used for periods up to two weeks.

The large amounts of PC and SPH in serum present the principal difficulty in utilizing one-dimensional TLC methods. At this concentration, these two lipid classes tend to trap the minor lipid classes. The use of the first solvent eliminated this problem and allows the observed separations. This method has also been successfully applied to phospholipid class separations of lipoprotein fractions, tissue samples, and bacterial samples in our laboratory.

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