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A technique for improved thin-layer chromatography of phospholipids

Although many systems utilizing thin-layer chromatography are available for separations of phospholipids by thin-layer chromatography, it has been very difficult to get good separations of a complex mixture of phospholipids on a single plate[1,2]. The present communication details methodology for improved resolution of components of such mixtures without prior laborious column separations before application to thin-layer plates.

In 1963, HORROCKS[3] reported several modifications of the stationary phase which improved phospholipid separation on thin-layer chromatograms. One of these was the addition of 20 ml of a saturated solution of sodium borate to 40 ml of water which was mixed with 30 g of Silica Gel G and applied to the thin-layer plate. This modification of the stationary phase was reported by these workers to give improved separation of phospholipids with a one-dimensional system of chloroform–methanol–ammonium hydroxide (65:24:4). In addition, several two-dimensional systems for the separation of phospholipids with various proportions of chloroform–methanol–ammonium hydroxide are also available in the literature[1,4,5]. The modification proposed in this paper involves the use of a stationary phase of Silica Gel G buffered with borate buffer at pH 8. Thirty grams of Silica Gel G were suspended in 60 ml of borate buffer (0.02 *M* sodium tetraborate, 3 ml; 0.02 *M* boric acid, 100 ml) and were made fresh each time plates were prepared. After the plates had been spread and dried at room temperature for several hours, they were activated at 100° for 1 h. The plates then could be stored over dessicant until use. Before being used, the plates had to be reactivated at 100° for 1 h.

Two solvent systems were tested for the separation of phospholipids on these plates, *i.e.* a one-dimensional system consisting of chloroform–methanol–water (65:25:4) and a two-dimensional system previously described by SKIDMORE AND ENTENMAN[4]. For the first dimension a system consisting of chloroform–methanol–7 *N*

TABLE I

R_F VALUES FOR TWO SYSTEMS FOR SEPARATION OF PHOSPHOLIPIDS

Adsorbent: SGG = Silica Gel G (E. Merck). SGG (borate) = Silica Gel G made up with borate buffer at pH 8. Solvent systems: I = Chloroform–methanol–7 *N* ammonium hydroxide, in the ratios (a) 60:35:5 and (b) 35:60:5. II = Chloroform–methanol–water (65:25:4).

Phospholipids	*Two-dimensional system*				*One-dimensional system*	
	SGG		*SGG (borate)*		*SGG*	*SGG (borate)*
	Ia	*Ib*	*Ia*	*Ib*	*II*	*II*
Cardiolipin	0.91	0.94	0.91	0.95	0.92	0.91
Phosphatidic acid	0.77	0.71	0.73	0.71	—	—
Phosphatidyl ethanolamine	0.65	0.60	0.60	0.60	0.83	0.75
Phosphatidyl serine	0.20	0.47	0.14	0.15	0.40	0.35
Phosphatidyl choline	0.36	0.36	0.35	0.30	0.60	0.53
Phosphatidyl inositol	0.30	0.64	0.17	0.66	0.30	0.24
Lysophosphatidyl choline	0.14	0.09	0.09	0.09	0.23	0.13

ammonium hydroxide (60:35:5) was used, while for the second dimension the system was chloroform–methanol–7 *N* ammonium hydroxide (35:60:5). With both solvent systems the buffered borate plates gave improved resolution of the phospholipid with less streaking and diffusion. This improved resolution enables one to separate quantitatively up to 200 μg of each of seven phospholipids. Total phospholipids were detected by immersing the entire plate in a closed container with iodine crystals and iodine vapors. Ninhydrin spray was used to detect amino nitrogen groups[6], and acid molybdate for phosphate[7], anthrone and diphenylamine[6] for glycolipid and Chargaff's reagent for choline[6].

Table I shows the R_F values (distance of center of spot from starting point divided by distance of solvent front from starting point) of seven phospholipids separated by the two-dimensional system and six phospholipids separated by the one-dimensional system. Figs. 1 and 2 show the comparison of thin-layer plates with the two-dimensional system only.

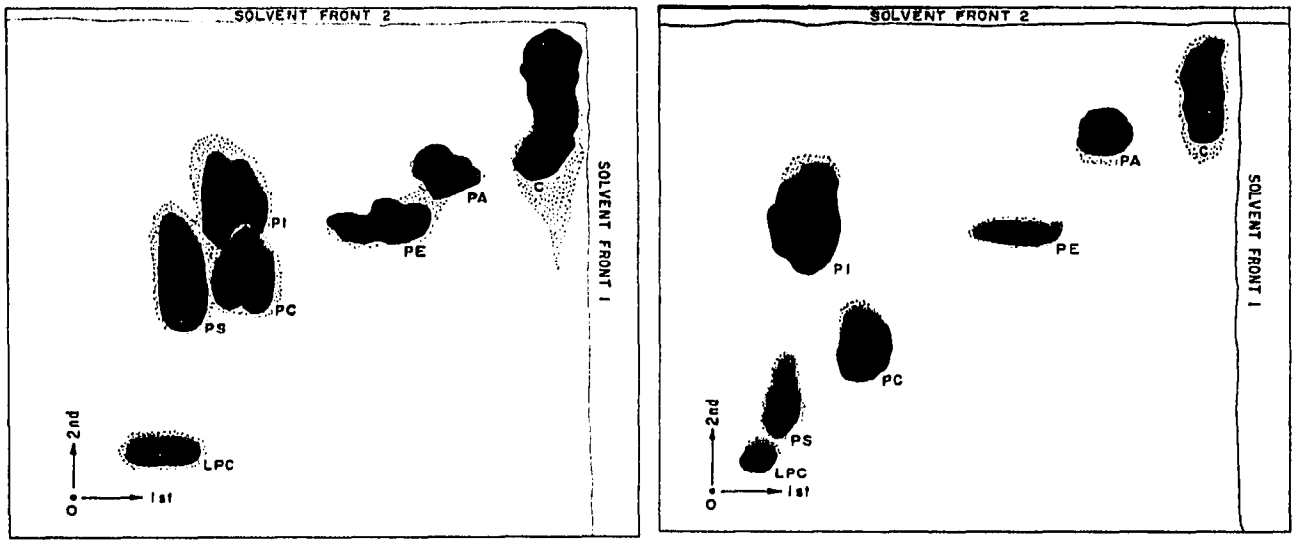

Fig. 1. Thin-layer chromatogram of phospholipid standards on Silica Gel G. The developing solvent in the first direction was chloroform–methanol–7 *N* ammonium hydroxide (60:35:5) and in the second, the ratio of the same solvent was 35:60:5. O = origin; LPC = lysophosphatidyl choline; PS = phosphatidyl serine; PC = phosphatidyl choline; PI = phosphatidyl inositol; PE = phosphatidyl ethanolamine; PA = phosphatidic acid; and C = cardiolipin.

Fig. 2. Thin-layer chromatogram of phospholipid standards on Silica Gel G in borate buffer at pH 8. The developing solvent in the first direction was chloroform–methanol–7 *N* ammonium hydroxide (60:35:5) and in the second, the ratio of the same solvents was 35:60:5. For abbreviations, see the legend to Fig. 1.

It may be seen from Table I that the plates buffered at pH 8 with borate buffer give better separation than those not buffered with both solvent systems for phosphatidyl serine, phosphatidyl inositol, and phosphatidyl choline. As shown on the tracings of the thin-layer plates (Figs. 1 and 2), much less diffusion of the spots occurs under conditions of borate buffering. This was confirmed by doing a recovery experiment. Samples of 200 μg of each phospholipid were applied to these plates and the phospholipids separated by the two-dimensional system. The spots were recovered

from the plates and analyzed for lipid[8] and phosphorus[9]. Calculation revealed that 95 % of the starting material was recovered.

An additional benefit of this method is that the complex mixture of phospholipids extracted from bacteria could be separated without the use of previous column separation and on a single plate. Other procedures required prior purification of the extract before adequate resolution could be obtained on the plates.

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Determination of inorganic radioiodide in ^{131}I-labelled compounds by means of thin-layer chromatography

Organic compounds labelled with a radioiodide for use in medical diagnosis or therapy generally contain inorganic radioiodide as their main impurity. According to current regulations, the radioiodide content should never exceed 5 %.

Several techniques are used for the determination of inorganic radioiodide, *viz.* paper chromatography, paper electrophoresis, thin-layer chromatography, precipitation, thin-layer electrophoresis, etc. Several authors, pharmacopeias, Atomic Energy Commissions and commercial firms such as Squibb, Abbott, Amersham, Hoechst, etc. have published on this topic[1–10].

Using thin-layer chromatography[11–12], we have devised a method for separating radioiodide from organic molecules[13–16]. We have tried to reduce the development time of the chromatogram and looked for readily available solvents for use with most of these compounds; 1 *N* hydrochloric acid was found to be the most suitable.