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## Note

# Effect of phosphor on thin-layer chromatography of phospholipids

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During the course of investigation of the separation of phospholipids on thin-layer chromatograms it was found that phosphor in the silica gel gave a different pattern of separation. With the previously reported[1] mobile phase ethanol–chloroform–triethylamine–water (35:30:30:8) changes in $R_F$ of several of the phospholipids were noted, with the greatest change in phosphatidyl serine.

This report describes the results obtained when silica gel plates from Whatman (LK 5 and LK 5F) and E. Merck (silica gel 60 and silica gel 60 $F_{254}$) were compared. It shows that different results were obtained when the silica gel contained phosphor. The changes in $R_F$ may be useful in qualitative identification studies.

## METHODS

The layers (20 × 20 cm) were scored into 10-mm lanes. On alternate lanes the phospholipids listed in Table I were applied. The mobile phase ethanol–

TABLE I

EFFECT OF PHOSPHOR ON PHOSPHOLIPID SEPARATIONS

All phospholipids were obtained from Avanti Biochemicals (Birmingham, AL, U.S.A.) except for ethandamine plasmalogen (brain), which was obtained from Supelco (Bellefonte, PA, U.S.A.).

| *Phospholipid* | $R_F$ *Whatman* | | *Merck* | |
|---|---|---|---|---|
| | *LK5* | *LK5F* | *Silica Gel 60* | *Silica Gel 60F254* |
| Cardiolipin | 0.78 | 0.70 | 0.73 | 0.69 |
| Dipalmitoyl phosphatidylglycerol (synthetic) | 0.74 | 0.71 | 0.64 | 0.65 |
| Phosphatidylinositol | 0.48 | 0.43 | 0.40 | 0.39 |
| Phosphatidylethanolamine | 0.58 | 0.50 | 0.38 | 0.31 |
| Dipalmitoyl phosphatidylethanolamine (synthetic) | 0.53 | 0.48 | 0.43 | 0.40 |
| Ethanolamine plasmalogen (brain) | 0.58 | 0.49 | 0.46 | 0.43 |
| Phosphatidyl serine (brain) | 0.48 | 0.16 | 0.29 | 0.12 |
| Oleoyl palmitoyl phosphatidylserine (synthetic) | 0.48 | 0.14 | 0.29 | 0.10 |
| Dipalmitoyl phosphatidylcholine (synthetic) | 0.30 | 0.25 | 0.15 | 0.17 |
| Phosphatidylcholine (brain) | 0.38 | 0.31 | 0.17 | 0.18 |
| Lysophosphatidylethanolamine | 0.24 | 0.20 | 0.26 | 0.27 |
| Sphingomyelin | 0.29 | 0.25 | 0.12 | 0.14 |

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chloroform–triethylamine–water (35:30:30:8) was allowed to migrate to 1 cm from the top of the layer in unlined tanks. The amount of phospholipid applied was 0.5–2 $\mu$g. The two plates of each brand were developed in the same tank.

After drying in air followed by final drying at 170°C for 2 min in an oven, the chromatograms were sprayed with 10% copper sulfate in 8% phosphoric acid until wet. The layers were dried in air for 5 min, at 120°C for 5 min and finally charred in an oven at 180°C for 10 min for detection.

## RESULTS AND DISCUSSION

The $R_F$ values found for the various phospholipids are listed in Table I. As indicated the phosphor included in the layer changes the values. Particularly notable is the case of phosphatidyl serine which in the normal layer travels above lecithin while in the fluorescent layer it travels below. This change can be of value in studies on qualitative investigation into the nature of separated phospholipids. It is also important to note that properties of difdesent silica gel layers can be somewhat different towards different compounds within a class.

## REFERENCE

1 J. C. Touchstone, S. S. Levin, M. F. Dobbins and P. C. Beers, *J. Liquid Chromatogr.*, 6 (1983) 179.