

## Modified spray for the detection of phospholipids on thin-layer chromatograms

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**SUMMARY** A simplified method for the preparation of the Dittmer-Lester spray for the detection of phospholipids on thin-layer chromatograms is described.

**KEY WORDS** modified spray · phospholipids · thin-layer chromatography · ammonium molybdate · mercury · components ratio · sensitivity · specificity

DITTMER AND LESTER (1) have described a sensitive and specific reagent, which is now widely used for the detection of phospholipids on chromatograms. The composition of the spray is based on that of the Zinzadze reagent (2) for the quantitative determination of phosphorus, which does not differ in principle from the more readily available reagent of Lucena-Conde and Prat (3). Accordingly, we have developed a similar spray for phospholipids that is highly specific and sensitive, simple to prepare, and stable during storage.

*Recommended Method of Preparation.* 16 g of ammonium molybdate is dissolved in 120 ml of water to give solution I. 40 ml of concentrated hydrochloric acid and 10 ml of mercury are shaken with 80 ml of solution I for 30 min to give, after filtration, solution II. 200 ml of concentrated sulfuric acid, followed by solution II, is added carefully to the remainder of solution I. The cooled mixture is diluted with water to 1 liter.

This spray reagent reacts with phospholipids on a thin-layer plate immediately, without heating, to give blue spots on a white ground.

*Investigation of the Effect of the Components Ratio.* Solutions I and II were prepared as described by Lucena-Conde and Prat (3). 16.3 g of ammonium molybdate was dissolved in 120 ml of water to make solution I. 50 ml of solution I, 25 ml of hydrochloric acid, and 5 ml of mercury were shaken for 10–15 min and the mixture was filtered to give solution II.

A portion of concentrated sulfuric acid was added carefully to a portion of solution I, a portion of solution II was

added, and the mixture was made up to 20 ml with water. The amount of solution I varied from 0 to 6 ml, of solution II from 0 to 8 ml, and of sulfuric acid from 0 to 13 ml. The various solutions obtained were used to spray a thin-layer silica gel plate to which spots of egg lecithin (1, 2, 5, 10, and 20  $\mu\text{g}$ ) had been applied.

The sensitivity of reagents, the coloration of the background, and the stability of spots and background were compared.

The reagent gave satisfactory staining over a wide range of Mo (VI), Mo (V), and  $\text{H}_2\text{SO}_4$  concentrations. Solution I alone does not stain phospholipids. Solution II alone produced an intense background, on which the phospholipid spots developed over some time. At low  $\text{H}_2\text{SO}_4$  concentrations, the two solutions produced a precipitate when mixed; at high concentrations, a dark background was obtained.

On the basis of these observations, the reagent composition given under *Recommended Method* is proposed. This reagent contains somewhat less molybdenum and sulfuric acid than that of Dittmer and Lester (1) and a much lower ratio of Mo (VI):Mo (V).

*Specificity.* Lecithin, phosphatidyl ethanolamine, and sphingomyelin were isolated from egg lipids by chromatography on alumina and silica gel. Phosphoinositide and cardiolipin were prepared from bakers' yeast (4). Sulfatide, psychosine, and sphingosine were kindly supplied by Dr. I. G. Zhukova (Institute of Organic Chemistry, Moscow). Fatty acids, cholesterol, glycerol 2- and 3-phosphates, fructose 6-phosphate, and adenosine-2'- (3')-monophosphate were commercial products.

The solutions of substances were applied to a thin-layer plate as spots of 5 mm diameter and sprayed with reagent. All the phospholipids listed produced a positive reaction in quantities of not less than 2  $\mu\text{g}$ ; all the others, as well as phosphoric acid and its salts, gave no color in quantities up to 50  $\mu\text{g}$  per spot.

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