

Spray detection of phospholipids on thin-layer chromatograms

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SUMMARY A new spray reagent with copper, ammonium molybdate, and sulphuric acid for the detection of phospholipids on thin-layer chromatograms is described. The specificity and sensitivity of the spray reagent is also shown.

SUPPLEMENTARY KEY WORDS ammonium molybdate
copper

THE DETECTION OF phospholipids on paper has been accomplished by means of various spray reagents (1), but the acidic ammonium molybdate spray (2) which has long been used for the detection of phospholipids on paper is not satisfactory when thin-layer silica gel plates are used. This is due to slow development of color at room temperature, to nonspecific reactions which occur during heating or longer development, or to some other reasons. Dittmer and Lester (3) have described a modification of the molybdenum blue reagent of Tzintzadze (4) for the detection of phospholipids on thin-layer chromatograms. Similarly, Vaskovsky and Kostetsky (5) have also described a modified spray reagent for phospholipids on

thin-layer chromatograms; it contains ammonium molybdate, mercury, hydrochloric acid, and sulphuric acid and is based on the more readily available reagent of Lucena-Conde and Prat (6). The phospholipid-specific spray described by Jatzkewitz and Mehl (7) is a combination of ammonium molybdate and vanadium-sulphuric acid reduced with zinc, and its use requires several steps and considerable manipulation. The spray reagent reported here is completely different from those previously described. Its usefulness cannot be ascribed to any known reactions with phosphorus, and the mechanism of its action is unknown at present.

Method of Preparation. 0.08 g of metallic copper (Allied Chemical Corp., General Chemical Div., New York) is placed in a solution of 0.25 g of ammonium molybdate (Mallinckrodt Chemical Works, St. Louis, Mo.) in 1 ml of distilled water. The mixture is chilled and 1 ml of concentrated sulphuric acid (Baker Analyzed Reagent, J. T. Baker Chemical Co., Phillipsburg, N.J.) is added; the deep blue solution is then shaken. This reaction mixture is kept for 2 hr at room temperature with occasional shaking. 40 ml of distilled water is then added and shaken; the color changes from deep blue to light brown. The copper metal is then removed and 3.2 ml of concentrated sulphuric acid is added; the resulting solution remains light brown.

This spray reagent gives satisfactory staining of phospholipids. The ammonium molybdate solution with or

without the concentrated sulphuric acid does not stain phospholipids.

Specificity. Lecithin, fatty acids, cholesterol, triglycerides, fructose-6-phosphate (all from Nutritional Biochemicals Corp., Cleveland, Ohio), glycerol-2- and 3-phosphates (Sigma Chemical Co., St. Louis, Mo.), phosphatidylethanolamine, phosphatidylserine, sphingomyelin, sphingosine, and cerebrosides were tested. Phosphatidylethanolamine, phosphatidylserine, sphingomyelin, and sphingosine were made available through the generosity of the Mental Health Research Institute, Ann Arbor, Mich.

The solutions to be tested were applied on precoated thin-layer-plate silica gel F-254 of 0.25 mm thickness (E. Merck A.G., Darmstadt, Germany, distributed by Brinkmann Instruments, Inc., Westbury, N.Y.) and sprayed with the reagent. The plate was then kept in an oven at 65–70°C for 5 min; it was removed and again sprayed with the reagent and kept for an additional 5–6 min in the oven. Phospholipids stained blue against a light blue background; all other compounds did not give any color. Overheating produced a pink coloration of the cholesterol, which ultimately turned greenish gray against a light blue background.

Sensitivity. The sensitivity of the phosphate spray was determined with commercial lecithin. The solution was applied to a thin-layer plate as spots of 4 mm diameter. The plate was developed with chloroform–methanol–water 65:24:4 (v/v/v), air dried, and then sprayed with the reagent; as little as 1 µg could be detected. This re-

agent is highly specific, sensitive, and very simple to prepare. It can be stored for a week or more in the cold.

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