

zu Fehlinterpretationen sein können), führt man die Elution und das anschliessende Verdunsten am besten in einem trockenen Stickstoffstrom durch. Die hierzu notwendige Glasapparatur ist nach der Fig. 2 leicht vom Glasbläser herstellbar. Ein weiterer Vorteil dieser Vorrichtung ist der Schutz labiler Substanzen vor Oxydation.

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Manganous chloride spray reagent for cholesterol and bile acids on thin-layer chromatograms

Cholesterol reacts with a variety of chemical substances to give colored products. KRITCHEVSKY¹ has summarized some of these color reactions of cholesterol. But the spray detection of cholesterol in thin-layer chromatography (TLC) has been accomplished by means of antimony trichloride², phosphomolybdic acid³, anisaldehyde-sulfuric acid⁴ and ferric chloride⁵. Antimony trichloride has its disadvantage due to its toxicity and reactivity with water to form insoluble precipitates. Phosphomolybdic acid and anisaldehyde-sulfuric acid gives a colored background and cannot clearly distinguish the cholesterol from the bile acids. All these difficulties may be overcome by the use of this manganous chloride spray reagent for the detection of cholesterol and bile acids on thin-layer chromatograms.

Materials and methods

Cholesterol and bile acids are obtained from Applied Science Laboratories, Pa., and manganous chloride from Allied Chemicals, General Chemical Division, N.Y. DuPont concentrated sulfuric acid is used. The samples are applied to Silica Gel F-254 of 0.25 mm thickness as supplied by Brinkmann Instruments, Westbury, N.Y., and detected without chromatography. The spray reagent is prepared by dissolving 50 mg of $MnCl_2 \cdot 4H_2O$ in 15 ml of water and 0.5 ml of concentrated sulfuric acid. After the reagent has been sprayed on the thin-layer plate, it is placed in an oven at 100-110° for 10-15 min and the color is noted. Five to ten micrograms of each in ethanol is sufficient for color detection.

Results and discussion

The color reactions of cholesterol and bile acids are shown in Table I. The

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TABLE I

COLOR REACTIONS OF CHOLESTEROL AND BILE ACIDS ON THIN-LAYER CHROMATOGRAM (10 μ g)

The thin-layer plate is sprayed with manganous chloride spray reagent ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 50 mg; water, 15 ml and concentrated sulfuric acid, 0.5 ml).

<i>Compound</i>	<i>Color</i>
Cholesterol	Pink
Cholic acid	Deep yellow
Chenodeoxycholic acid	Greyish green
Deoxycholic acid	Yellow
Hyodeoxycholic acid	Tan
Lithocholic acid	Light pink

color of cholesterol begins to fade and the bile acid colors deepen after 5 min. On longer exposure to air, all the characteristic color disappears. So, the cholesterol color should be noted after removing the plate from the oven and the bile acids after 5 min from then. There is no background color and so the resulting color stands out clearly from the white background. This method allows as little as 1 μ g of cholesterol and 2 μ g of bile acids to be detected and the reagent is very simply to prepare. It is always suggested to prepare ones' own color standards.

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