Ferric chloride spray detector for cholesterol and cholesteryl esters on thin-layer chromatograms

ROBERT R. LOWRY

Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331

SUMMARY The use of a stable solution of ferric chloride for the detection of cholesterol and cholesteryl esters on thin-layer chromatographic layers is described. Its advantages over the commonly used Carr–Price reagent are listed, and the levels of sensitivity for the new reagent are shown. Detection of other lipid classes is also possible.

KEY WORDS thin-layer chromatography · cholesterol · cholesteryl esters · detection · ferric chloride spray · sensitivity levels

ANTIMONY TRICHLORIDE IS USED EXTENSIVELY in thin-layer chromatography as a detection agent for cholesterol and sterols in general. For example, Bobbitt (1) lists some 16 references under sterols where the Carr–Price reagent, or a modification of it, was used for detection purposes. Its major disadvantages are its toxicity (as well as that of the solvent usually used with it, carbon tetrachloride) and its reactivity with water to form insoluble precipitates. The latter property is particularly annoying when a spraying device is used in which moisture can condense in the capillary passages.

For several years in our laboratory we have used the colorimetric method of Zlatkis, Zak, and Boyle (2) as modified by Chiamori and Henry (3) to determine cholesterol. Their method makes use, in part, of the property of ferric chloride to intensify the color obtained in the reaction of sterols with sulfuric acid. This method, if adapted for thin-layer chromatography, should give a procedure that would be reasonably specific for cholesterol and its esters and also eliminate the hazards and difficulties associated with the Carr–Price reagent.

Experimental Details and Results. The reagent was prepared by dissolving 50 mg of $FeCl_3 \cdot 6H_2O$ in 90 ml of water and adding 5 ml of glacial acetic acid and 5 ml of concentrated sulfuric acid. This reagent, in dilute acid, is stable at room temperature for over 3 months. We believe that the usual instability of ferric chloride reagents is due to a reaction with the concentrated acids used as the solvents.

After the reagent has been sprayed on the layer, the plate is heated at 100°C to develop the characteristic red to violet color, which appears within 2–3 min. The color is not stable and if exposed to air it will continue to darken gradually. Color development proceeds more rapidly with the free cholesterol than with its esters. This difference can be used in conjunction with the R_f values to establish the identity of the spots obtained. Because of the sulfuric acid present, continued heating causes charring of other organic material and the reagent can therefore also be used as a general detection reagent.

Cholesterol and cholesteryl palmitate standards of >99% purity were obtained from The Hormel Institute, Austin, Minn. The limits of detection for these compounds were 1.09 ± 0.06 $\mu g/cm^2$ (n = 8) and 1.21 ± 0.14 $\mu g/cm^2$ (n = 7), respectively, as viewed in visible light. Under UV light, which increases the sensitivity of detection, as shown by Nelson and Booth (4), the levels of detection were increased by a factor of approximately 10. The samples were applied to Silica Gel G microplates of 100μ thickness as supplied by Schaar Scientific Co., Chicago, Ill., and detected without chromatography.

Manuscript received 21 September 1967; accepted 12 February 1968.

REFERENCES

1. Bobbitt, J. M. 1963. Thin-Layer Chromatography. Reinhold Publishing Corp., New York. 169–174.
2. Zlatkis, A., B. Zak, and A. J. Boyle. 1953. *J. Lab. Clin. Med.* **41:** 486.
3. Chiamori, N., and R. J. Henry. 1959. *Am. J. Clin. Pathol.* **31:** 305.
4. Nelson, G. J., and R. A. Booth. 1967. *Anal. Biochem.* **20:** 198.

ADDENDUM

Journal of Lipid Research, Volume **9,** Number 3, May, 1968, page 397.

At the end of his article the author requests insertion of the following.

> After the publication of the above Note, an earlier description of a ferric chloride spray for the detection of free sterols on thin-layer chromatograms came to my attention (J. R. Claude. 1966. *J. Chromatog.* **23:** 267). My estimate of the sensitivity of this reagent, while more precise than Claude's, is in good agreement with his.