An improved spray reagent for detection of bile acids on thin-layer chromatoplates

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SUMMARY A spray reagent containing 8-hydroxy-1,3,6-pyrenetrisulfonic acid sodium salt is described, which allows detection of as little as 1 μg of bile acids under long-wave UV light. The bile acids can be eluted directly from the sprayed plates with acetone without eluting the spray reagent.

SUPPLEMENTARY KEY WORDS 8-hydroxy-1,3,6-pyrenetrisulfonic acid • ultraviolet light

NONDESTRUCTIVE methods for detecting bile acids on thin-layer chromatographic plates include the use of water spray (1), iodine vapor or spray (1), and the use of a pyrene spray reagent to detect bile acids as fluorescent spots (2). The latter system works well but requires the immediate redevelopment of the sprayed plate to remove the pyrene before analysis of the detected bile acids can be achieved.

Use of the reagent 8-hydroxy-1,3,6-pyrenetrisulfonic acid trisodium salt (Eastman P7281; Eastman Organic Chemicals, Rochester, N.Y.) instead of pyrene as a spray reagent yields similar fluorescent spots from bile acids on a quenched background, but has the advantage that nonconjugated bile acids can be eluted from the silica gel with acetone while the reagent remains firmly bound to the silica gel; this eliminates the second development necessary when using unsubstituted pyrene. This reagent does not interfere with the detection of fluorescent-absorbing materials such as fatty acids when Silica Gel GF plates are viewed under short-wave UV light, but shows as little as 1 μg of bile acids as light blue spots on a dark purple background when viewed under long-wave UV light. The spray will also detect bile acid conjugates, neutral sterols, and fatty acids. The spray is colorless and does not interfere with visual light inspection.

We dissolve 5 mg of the reagent in 100 ml of redistilled methanol. The reagent is stable under laboratory conditions for at least 8 weeks. After spraying, the plates are dried and inspected under long-wave fluorescent light (3600 A). The fluorescent area can be removed, and the bile acid eluted with a few milliliters of acetone. Although the dye has an apparent R_f of 0.0 when spotted on a TLC plate and developed with acetone, we can

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occasionally detect small fluorescence when respotting eluted bile acid samples. This dye may be eluted due to deactivation of the silica gel. This can be prevented in our hands by placing a few millimeters of activated silica gel under the recovered silica gel before elution.

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