Test for Radiochemical Purity of Sodium Radio-Iodide (I^{131}) Solution U.S.P. by Thin-Layer Chromatography

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A thin-layer chromatography method for separating inorganic iodide and iodate ions is described. The method is useful in establishing the radiochemical purity of sodium radio-iodide (I^{131}) solution U.S.P.

THE EXTENSIVE use of sodium radio-iodide (I^{131}) solution U.S.P. XVI in the diagnosis and treatment of disease prompted the report of results on the use of thin-layer chromatography (TLC) in testing for the radiochemical purity of this preparation. As originally produced, either as a uranium fission product or by neutron bombardment of tellurium, the I^{131} may exist in any of its oxidation states and may be mixed with other elements and compounds. Even though after extensive purification the iodine may be freed from other contaminants and be reduced essentially to iodide, these preparations may still contain a substantial amount of I^{131} in the form of iodate if a reducing agent stabilizer, such as cysteine, is not present. Preparations containing iodate give erroneous and inconsistent clinical results (1, 2). The U.S.P. has placed a limit of 5% on the iodate content of sodium radio-iodide (I^{131}) solution.

TLC has great utility in quality control procedures because of its separatory efficiency, rapidity, simplicity, and relatively low cost. The U.S.P. paper chromatography method used for separating iodide and iodate ions in determining the radiochemical purity of I^{131} takes about 5 hr. Using a TLC procedure which gives equally good separation, the entire test can be completed in about 1 hr. This radiopharmaceutical has been used traditionally to study the aspects of radiochemical purity in courses dealing with radioisotope techniques. The TLC method discussed here enables the student to complete the radiochemical purity test in one laboratory session.

EXPERIMENTAL AND RESULTS

Plates were prepared with a slurry of 17 Gm. of silica gel G[1] for thin-layer chromatography, stirred with 34 ml. of distilled water for 1 min., then poured into the applicator[1] and spread on glass plates (5 × 20 cm.). The prepared plates were dried in air for about 30 min. and activated in an oven at 110–115° for 1 hr., then immediately cooled and stored in a desiccator over calcium chloride.

Standard solutions containing unlabeled potassium iodide (0.1%) and potassium iodate (0.2%), dissolved in 1% w/v sodium bicarbonate solution, were used as reference substances. Five microliters of each solution was applied separately and as mixture at points 2 cm. from the lower edge of the plate. One microliter of sodium radio-iodide (I^{131}) solution U.S.P.,[2] representing about 30,000 c.p.m. was applied to one point containing the mixture of reference substances. The radioactive solution used previously had been found to be radiochemically pure (contained only radioactive iodide) determined by the U.S.P. paper chromatography method and by autoradiography using Kodak no-screen X-ray film. Sufficient developing solvent (75% methanol) was placed in the bottom of the chamber to insure a 1-cm. layer of the solvent. Time of development (when the solvent front reached a line marked 12 cm. from the point of application) was about 40 min.

The location and intensity of the radioactive zones on the developed plates were determined with a Nuclear-Chicago Actigraph thin-layer radiochromatogram scanner (model 1036). Prior to scanning, a small amount of radioactive ink was spotted near the lower edge of the plate to serve as a marker for the recorder tracing (Fig. 1). The TLC plates were scanned at a speed of 0.75 in./min. using a collimator slit width of 0.25 in.

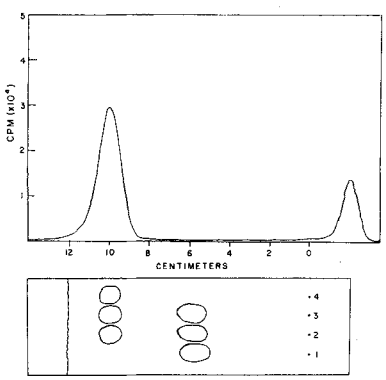

Fig. 1.—TLC plate (lower) and scan (upper) for radioactivity. Key: 1, iodate; 2, iodide and iodate; 3, iodide, iodate, and radio-iodide (I^{131}); 4, iodide. (See text.)

The plates were then sprayed with a 0.2% w/v solution of ascorbic acid, then with starch T.S. The iodate zone gave an immediate blue color. The plate then was sprayed with a 5% w/v solution of hydrogen peroxide, whereupon the iodide zone rapidly appeared as a blue spot. The R_f of iodate was about 0.50, while the R_f of iodide was about 0.83. The zones were well separated and circular in shape at these concentrations. The spots on the chromatograms were marked as soon as they appeared, since they fade a few minutes after spraying. The reagents readily detected 5 mcg. of iodide and 10 mcg. of iodate.

SUMMARY

A TLC method for establishing the radiochemical purity of sodium radio-iodide solution U.S.P. was

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[1] Purchased from Research Specialties Co., Richmond, Calif.

[2] Purchased from Nuclear Consultants Corp., St. Louis, Mo.

studied. The method provides rapid and effective separation of iodide and iodate ions and should serve as a suitable technique for the quality control of radiopharmaceuticals containing I^{131}.

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8-Hydroxyquinoline Sulfate as a Preservative for Tuberculin PPD (Mantoux)

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The preservative 8-hydroxyquinoline sulfate (8-HQS) has been tested for its antimicrobial activity in solutions of tuberculin purified protein derivative (PPD). All tests were carried out at room temperature (18–22°). Two yeast and three mold species were destroyed readily by 0.01 per cent 8-HQS. The preservative also was effective against small inocula (approximately 10–100 cells) of *Pseudomonas aeruginosa*, whereas large inocula (1000 cells) usually grew out. Four strains of *Staphylococcus aureus* died quickly in the solution with or without the presence of 8-hydroxyquinoline sulfate.

TUBERCULIN PURIFIED protein derivative (PPD) is a specific protein isolated from the culture filtrate of *Mycobacterium tuberculosis*. Introduced intracutaneously or percutaneously, it produces localized induration and swelling in a person who has had an infection or is currently infected with *M. tuberculosis*. Intracutaneous testing with tuberculin solutions of specified strength is used widely as part of tuberculosis prevention programs.

PPD is prepared as a concentrated stock solution preserved with phenol. For intracutaneous use (Mantoux test), the stock solution is diluted to contain graded amounts, usually 0.2, 1, 2, 20, and 50 mcg. of PPD per milliliter. Phenol, however, reduces the potency of diluted PPD solutions (1, 2); for this reason, Magnusson *et al.* (3) devised a diluent for PPD which contains 8-hydroxyquinoline sulfate[1] (8-HQS) as a preservative.

Although 8-HQS has been used for many years in the diluent for PPD, there have been no reports concerning its efficacy as a preservative for this material. However, when 8-HQS was used as a preservative for antimeasles serum, the serum became contaminated with bacteria; two children who received the contaminated serum died, while a third became ill and recovered (4). There is no information regarding the concentration of 8-HQS used. In the same report, Olin and Lithander described the results of injecting a contaminated solution of tuberculin which contained no preservative. Of the 11 children given Mantoux tests with the contaminated material, all showed symptoms of toxicity, and one died. In both of these incidents, *Staphylococcus aureus* was the contaminant.

The reported failure of 8-HQS to prevent the growth of contaminants in antimeasles serum and the ability of contaminants to grow in tuberculin in the absence of a preservative suggested that an investigation of the efficacy of 8-HQS in PPD should be made. This report gives the results of such an investigation.

MATERIALS AND METHODS

PPD.—PPD prepared as a concentrated stock solution (5) was diluted to contain 50 tuberculin units (TU) per milliliter; 1 TU = 0.00002 mg. protein.

WHO Diluent.—The diluent devised by Magnusson *et al.* (3) was used for all preparations. This diluent is a phosphate buffered saline (1.45 Gm. KH_2PO_4, 7.6 Gm. $Na_2HPO_4 \cdot 2H_2O$, 4.8 Gm. NaCl in 1.05 L. of distilled water) containing 0.005% polysorbate 80[2] and 0.01% 8-hydroxyqinoline sulfate (8-HQS). For some experiments, diluent was made without 8-HQS.

Cultures.—The source of the cultures, methods for preparation of inoculum, and inoculation of preserved solutions with challenge organisms have been described previously (7). The only deviation from these methods was a triple washing of inoculum and the resuspension with Magnusson's (WHO) diluent, from which the 8-HQS was omitted.

Testing of Preservative Activity.—Inoculated solutions were incubated at room temperature (18–22°) and subcultured with a standard loop to Difco nutrient broth for bacteria or Difco Sabouraud agar for yeasts and molds. When quantitative results were desired, 0.1-ml. quantities of the preserved solutions or dilutions thereof were spread on suitable agar media in Petri dishes.

8-Hydroxyquinoline Sulfate.—8-Hydroxyquinoline sulfate (Eastman Organic Chemicals, 1776) was used. The method for analysis was developed in these laboratories. Because it has not been published previously in detail, it is described as follows.

Spectrophotometric Determination of 8-Hydroxyquinoline Sulfate

Principle.—The method is based upon the characteristic light absorption which 8-HQS shows in the ultraviolet region with an absorption maximum at 240 mμ. At this wavelength and in the

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[1] Marketed as Chinosol.

[2] Marketed as Tween 80 by Atlas Chemical Industries, Wilmington, Del.