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PURIFICATION AND RADIOCHEMICAL QUALITY CONTROL OF ^{131}I-19-IODOCHOLESTEROL

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Radiochemical quality control and purification techniques were developed for ^{131}I-19-cholesterol. The amount of ionic ^{131}I-iodide is rapidly determined using instant thin-layer chromatography. Samples of the labeled steroid that contain greater than 8% of ionic ^{131}I-iodide are purified with one of two techniques, both using a weakly basic ion-exchange resin. The first technique, a batch ion-exchange process, is suitable for removing relatively low concentrations of ionic ^{131}I-iodide; if higher concentrations of this radiochemical contaminant are present, the second or column technique is more suitable.

Use of ^{131}I-labeled 19-iodocholesterol as a tumor-localizing agent has been reported (*1*) and is under investigation in select patients at several medical centers. The material labeled through isotope exchange is, however, furnished as a radiochemical rather than a radiopharmaceutical. Conversion to the latter form indicates a need for quality control including, in part, steps to be sure the material is sterile, pyrogen-free, and is radiochemically pure. Sterilization through the use of Millipore membrane filters and pyrogen checks presents no problem. But we have found it necessary to develop steps to routinely check the radiochemical purity and to develop a purification technique since some samples received have contained up to 20% ionic ^{131}I-iodide. This iodide probably forms primarily because of temperatures involved in transportation of the material. We have for almost 2 years used the described purification techniques with excellent results.

METHODS AND RESULTS

Purity checks. The radiochemical manufacturer (*2*) uses thin-layer chromatography with an ethanol/chloroform solvent system to check for the presence of ionic iodide. It is necessary to "load" the chromatography system by adding stable ionic iodide to the material that is to be assayed. Also, a considerable time is necessary to develop the chromatogram sufficiently for assay depending on solvents used, temperature, and extent of development. This may be several hours. Because of this we prefer to use the following more rapid technique to determine the ionic iodide content of the radiochemical: Gelman Silica Gel (SG) ITLC sheets cut into 1.5- × 20-cm strips are conditioned by drying in a 110°C oven for 30 min and are then stored until use in a desiccator that contains drierite as the desiccant.

In use, 2–5 μl of the labeled iodocholesterol are spotted about 1½ cm from the bottom of the ITLC strip. The strip is quickly dried in a stream of nitrogen and the chromatogram is then developed ascendingly at room temperature using a solvent composed of 50:50 methanol and 10% aqueous ammonium acetate solution. The ITLC is then air-dried and 0.5-cm increments are radioassayed. Iodocholesterol is retained near the application site (Rf 0.10) whereas the ionic iodide migrates almost with the solvent front (Rf 0.95). Total time for spotting, developing, and assaying the radiochromatogram is less than 40 min. Ten simultaneous chromatograms conducted on a radiochemically impure sample of

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labeled iodocholesterol gave a percent purity value of 87.2 with a standard deviation of ±2.1%.

Purification. Iodine-131-labeled iodocholesterol, which by ITLC quality-control checks contains more than 8% ionic ^{131}I-iodide, is purified by one of the two following techniques depending on the amount of ionic iodide to be removed. The supplier, at the start of the studies, stated this impurity level was the maximum to be tolerated in the interest of patient dosimetry.

Batch technique. The batch technique consists of mixing ion-exchange resin and iodocholesterol solution, allowing equilibrium to be established, and then withdrawing the solution.

Two and one-half grams of AG3-X4A weakly basic ion-exchange resin (Bio-Rad Laboratories, Richmond, Calif.) are placed in the chamber of a clean pyrogen-free Wheaton-Hopkins tagging vial which is fitted with a coarse fritted disk (Wheaton Scientific Company, Millville, N.J.). The vial is stoppered and crimped at both ends, a venting needle (no. 25 × ⅝ in.) is inserted in the top septum (on the end farthest from the sintered disk), and the resin is rinsed with five 10-ml aliquots of sterile, pyrogen-free, isotonic saline solution. The saline is added through the top septum and withdrawn from the bottom end through the sintered glass filter using a hypodermic syringe. The vial is shaken vigorously before withdrawing each wash solution. After the final wash has been withdrawn, the venting needle is removed and the vial and resin contents are sterilized by steam autoclaving. After cooling, the sterilized vial is stored in a large stoppered test tube until usage. In use, the vial is placed in a lead pig and the labeled iodocholesterol solution is added through the top septum using a shielded hypodermic syringe. The vial and contents are shaken and then allowed to stand for 10 min for equilibration to occur. The purified ^{131}I-labeled iodocholesterol is withdrawn through the filtered (lower) end with a hypodermic and is placed in a sterile, pyrogen-free, evacuated vial. The ion-exchange resin is rinsed with 2 ml of sterile, pyrogen-free, isotonic saline solution containing 1.6% Tween 80 and 6.0% ethanol. The rinsings are withdrawn through the filter and are also added to the purified iodocholesterol solution.

The batch technique removes up to 80% of the ionic iodide, depending on the equilibrium established with the resin. Triplicate samples of impure iodocholesterol containing about 20% ionic ^{131}I-iodide as contaminant, after being treated using the batch technique, were found to possess a radiochemical purity of 94 ± 2%. Six samples containing about 12–15% ionic iodide after purification were found to be 96 ± 1.5% pure.

Column technique. The column technique involves passing the impure iodocholesterol through an ion-exchange column. The column performs as a series of batch separations since the column may be visualized as an incremental succession of equilibria (*3*). This results in more efficient removal of ionic iodide. Two and one-half grams of AG3-X4A ion-exchange resin, presoaked in sterile, pyrogen-free, isotonic saline solution, are placed in a 1-cm diam chromatographic tube resulting in a column about 10 cm long. The column is washed with five 10-ml portions of sterile, pyrogen-free, isotonic saline solution. After the last rinse, the column is allowed to drain dry.

In use, the column is placed behind a lead shield and the impure, labeled iodocholesterol is carefully placed on the column using a shielded hypodermic syringe fitted with a 3½-in. needle. The solution is passed slowly through the column bed (at a flow rate of about one drop per 1–3 sec) and the eluate is collected in a clean flask. The column is then rinsed with 2 ml of sterile, pyrogen-free, isotonic saline solution containing 1.6% Tween 80 and 6% ethanol. This rinse is also collected in the flask. Using a hypodermic syringe, the flask contents are sterilized by passing them through a 0.22-micron Millex Millipore membrane filter and are stored in a sterile, pyrogen-free, evacuated flask. Ten iodocholesterol samples, each containing about 18–20% ionic ^{131}I-iodide impurity, after undergoing the ion-exchange procedure were found to be 98.5 ± 1.0% radiochemically pure.

DISCUSSION

The ITLC system used shows excellent reproducibility with chromatograms performed on the same solution. Gelman SA (silicic acid) ITLC also has been used and gives somewhat similar results. However, because of a faster development rate, SG strips are preferred.

Regarding the purification step, it was found that if strong basic ion-exchange resins are used (such as Rohm and Haas IRA-400 or Dow Chemical Dowex 1-X8), degradation of the labeled molecule occurs due to the strong ionic nature of the resin. With the weaker basic ion-exchange resin used, no major degradation of the ^{131}I-labeled iodocholesterol occurs and pure samples of the labeled radiopharmaceutical can be passed through the resin with little or no loss of activity.

REFERENCES

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