

Quality Control Procedures for Newer Radiopharmaceuticals

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This is the fourth article in a four-part series on new radiopharmaceuticals. Upon completion of this article, the reader should be able to (1) recognize the need for assessment of radiochemical purity, (2) describe some of the available systems, and (3) be aware of common errors in performing quality control on the newer radiopharmaceuticals.

Because radiopharmaceuticals are intended for human administration, quality control procedures are essential in ensuring the safety of these preparations. Although extensive quality control procedures are performed by the manufacturer, many radiopharmaceutical preparations are prepared in nuclear medicine departments using kits and short-lived radionuclides including technetium-99m (^{99m}Tc) and indium-111 (^{111}In). As a result, the ultimate responsibility for quality assurance of radiopharmaceuticals lies with the nuclear medicine department, usually with the radiopharmacist or the nuclear medicine technologist responsible for the nuclear pharmacy.

Radiopharmaceuticals, whether commercial or in-house preparations, must be subjected to physicochemical and biological testing. Physicochemical testing includes the examination and determination of the physical state, osmolality, pH, chemical purity, radionuclidic purity, and radiochemical purity of the radiopharmaceutical. Biological testing of radiopharmaceutical preparations includes sterility and pyrogenicity testing.

Radiochemical purity is defined as the proportion of the total radioactivity that is present in the specified chemical form. Numerous methodologies can be employed to assess the radiochemical purity of radiopharmaceuticals including thin layer chromatography, paper chromatography, gel permeation chromatography, high performance liquid chromatography (HPLC), and gel electrophoresis. Since time is critical in a nuclear medicine department, radiochemical quality control procedures must be rapid and relatively easy, in order to gain the maximum amount of information in the minimum amount of time. Thus, this review is written with an emphasis on rapid radiochemical quality control procedures for newer radiopharmaceuticals. The quality control procedures outlined in the text are fairly easy to use and have proved reliable in clinical nuclear medicine.

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MINIATURIZED CHROMATOGRAPHY PROCEDURES

Miniaturized chromatography procedures for determining the radiochemical purity of existing radiopharmaceuticals, including ^{99m}Tc (1-5) and iodinated radiopharmaceuticals (6), have been described in the nuclear medicine literature. As shown in Figure 1, chromatography strips, consisting of various support media, are cut into small sizes (0.7×6 cm or 0.7×8 cm). Origin, cut, and solvent front lines are drawn on each strip. The location of the cut line is dependent on the migration of the specific radiopharmaceutical. Chromatographic procedures include spotting the preparation on the origin line of the respective strips and developing the strips in the appropriate solvent system. Following solvent migration to the solvent front line, the strips are removed from the solvent system, cut, and assayed for activity using a well detector or dose calibrator.

Common Errors Associated with Miniaturized Chromatography

Although miniaturized chromatography procedures have been used for many years with few problems, a few procedural errors can and do occur (7,8). Some of these are listed in Table 1. The two most frequent errors are positioning the strip so that the radiopharmaceutical spot on the strip is below the initial solvent level in the developing vials and not counting the strips correctly. Because procedural errors can result in extremely inaccurate assessments of radiochemical purity, careful technique must be utilized with these chromatographic procedures.

MINIATURIZED CHROMATOGRAPHY PROCEDURES FOR NEWER RADIOPHARMACEUTICALS

Iodinated Radiopharmaceuticals

Miniaturized chromatography systems for iodinated radiopharmaceuticals including radioiodinated monoclonal antibodies (Mab) and iodine-123 iodoamphetamine [^{123}I]IMP (9-11) are listed in Table 2. Specific cut lines on chromatography strips are drawn at Rf values of 0.5. With these chromatography systems, the specific unbound radionuclides migrate with the solvent front (Rf = 1.0), whereas the bound radiopharmaceuticals remain at the origin (Rf = 0.0).

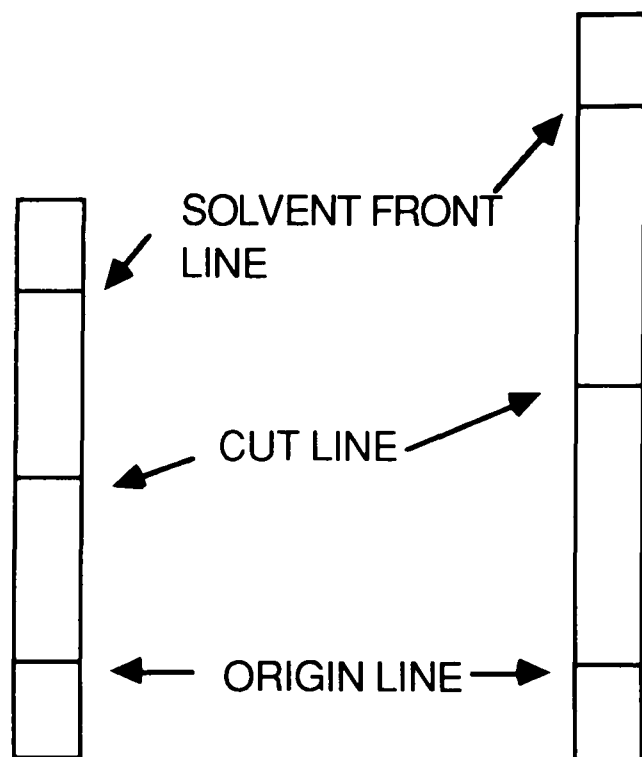


FIG. 1. Typical miniaturized chromatography strips (0.7 × 6.0 cm on left and 0.7 × 8.0 cm on right) showing origin, solvent front, and cut lines.

Indium-111 Radiopharmaceuticals

The miniaturized chromatography system for ¹¹¹In-labeled Mab is listed in Table 3. Prior to chromatographic evaluation, unbound ¹¹¹In is converted to the DTPA chelate by mixing 50 μl of ¹¹¹In-labeled Mab with 25 μl of 0.05 M DTPA for 1 min (12). After solvent migration, ¹¹¹In-DTPA chelate migrates with the solvent front ($R_f = 1.0$), whereas ¹¹¹In-labeled Mab remains at the origin ($R_f = 0.0$). Typical chromatography strip activity distributions of ¹¹¹In-DTPA chelate and ¹¹¹In-antimyosin Mab (Myoscint, McNeil Pharmaceutical, Spring House, PA) are found in Figure 2.

Technetium-99m Radiopharmaceuticals

Miniaturized chromatographic systems for newer ^{99m}Tc radiopharmaceuticals, all of which are currently used in our nuclear medicine facility, are shown in Table 4. Some of the chromatography systems mentioned in Table 4 have been developed in our laboratory or other laboratories (13–15), while some of these systems are modifications of the manufacturers' recommended quality control procedures. These systems were designed to be faster and/or easier to use than the specific manufacturers' chromatography systems. Specific chromatography systems for newer ^{99m}Tc radiopharmaceuticals are described below.

Technetium-99m Exametazime (Ceretek™). Ceretek is manufactured by Amersham Corp., Arlington Heights, IL. An excellent review of the manufacturer's recommended

TABLE 1. Common Errors or Pitfalls Associated with Miniaturized Chromatography Systems

Source of Error	Result
Origin, where strip spotted, is below the initial solvent level in the developing vial.	Activity will distribute throughout the entire chromatography strip resulting in inaccurate results. Spot new strip correctly.
Strips are counted too close to the NaI(Tl) well detector.	Dead time of crystal may be excessive resulting in gross overestimation of percent activity associated with the lower activity section of the strip. Increase distance of strips from detector, which will reduce dead time.
Strips are counted in dose calibrator.	Insensitivity of dose calibrator may result in large errors when counting low activity strips. If possible, spot more radiopharmaceutical activity on strip prior to developing.
Chromatography strips and solvents are too old.	Migration pattern of radiopharmaceutical may be changed. Also streaking of activity may occur. These can lead to erroneous results. Use new solvents and dry strips prior to use.
Strips and/or solvents reversed.	Totally inaccurate results may be obtained. Repeat entire QC procedure.
Radiopharmaceutical spot is dried prior to solvent development.	Oxidation of radiopharmaceutical may occur. Also binding of radiopharmaceutical with support media may result. Results in inaccurate assessment of radiochemical purity. Repeat entire QC procedure.
Strip is eluted past solvent front line.	If strip is eluted significantly past the solvent front line, the cut line must be changed to maintain the same R_f value.

three-strip chromatography procedure is found in the literature (8). Our laboratory has developed a single-strip miniaturized chromatography system to separate the ^{99m}Tc lipophilic fraction ($R_f = 1.0$) from other ^{99m}Tc radiochemical impurities ($R_f = 0.0$) (14). The chromatography system is similar to a system cited in the literature (16), which utilizes paper chromatography with diethyl ether as the solvent. Our proposed chromatography system consists of Whatman 17 strips (Whatman Chromatography Products, Clifton, NJ) with ethyl acetate as the solvent. A typical activity distribution profile of ^{99m}Tc exametazime and free ^{99m}Tc pertechnetate on a developed Whatman 17 chromatography strip is shown in Figure 3. The single-strip chromatography system is very rapid, taking less than 1 min to complete, and it is faster and

easier to use than the three-strip chromatography method recommended by the manufacturer (17). With the single-strip system outlined above, it is imperative that the strip be placed in the solvent *immediately* after spotting; failure to do so will result in underestimating the ^{99m}Tc lipophilic component. It should also be emphasized that the single-strip chromatography system separates the lipophilic fraction from *all* other radiochemical impurities so quantitation of specific radiochemical impurities is not possible.

Technetium-99m Teboroxime (CardioTec™). CardioTec is manufactured by Squibb Diagnostics, New Brunswick, NJ. The chromatography system described in Table 4 is a miniaturized version of the system recommended by the manufacturer (18).

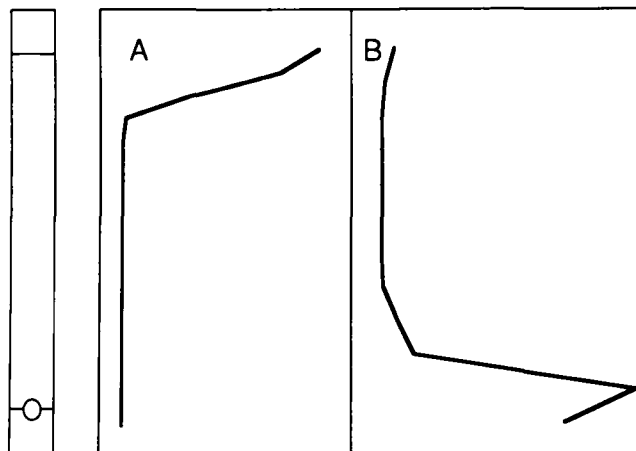


FIG. 2. Typical chromatography strip activity distribution of (A) ^{111}In -DTPA and (B) ^{111}In -labeled monoclonal antibody (Myoscint) in chromatography system consisting of ITLC-SG with normal saline.

TABLE 2. Miniaturized Chromatography Procedures for Iodinated Radiopharmaceuticals

Compound	Support Media	Solvent	Rf Values
Iodine	ITLC-SG	Acetone	Iodide = 1.0 Iodate = 0.0 Periodate = 0.0
Iodinated Mab	ITLC-SG	Normal saline	Iodinated Mab = 0.0 Free iodide = 1.0
^{123}I]IMP	ITLC-SA	10% NaCl	Unbound ^{123}I = 1.0 ^{123}I]IMP = 0.0

TABLE 3. Miniaturized Chromatography Procedures for Indium-111 Radiopharmaceuticals

Compound	Support Media	Solvent	Rf Values
^{111}In Mab (DTPA challenge)	ITLC-SG	Normal saline	^{111}In Mab = 0.0 ^{111}In -DTPA = 1.0

By miniaturizing the system, strip developing time has been significantly reduced from approximately 20 min for the manufacturer's recommended strips to less than 3 min for the miniaturized strips. At the same time, maximal separation between ^{99m}Tc impurities (pertechnetate and hydrolyzed reduced) and ^{99m}Tc teboroxime has been maintained. A chromatography strip activity distribution profile of a typical ^{99m}Tc teboroxime preparation is shown in Figure 4. Migration of the radiopharmaceutical occurs in the chromatography system consisting of Whatman 31ET strips with acetone/saline (1:1); separation of free pertechnetate from ^{99m}Tc teboroxime occurs in the system consisting of Whatman 31ET with saline.

Technetium-99m Sestamibi (Cardiolite™). Cardiolite is

TABLE 4. Miniaturized Chromatography Procedures for Technetium-99m Radiopharmaceuticals

Compound	Support Media	Solvent	Radiochemical Impurities Detected
^{99m}Tc Mab	ITLC-SG	Normal saline	Free ^{99m}Tc pertechnetate
^{99m}Tc Ceretec (Single strip)	Whatman 17	Ethyl acetate	Free ^{99m}Tc pertechnetate ^{99m}Tc hydrolyzed reduced ^{99m}Tc lipophobic fraction
^{99m}Tc CardioTec	Whatman 31ET Whatman 31ET	Normal saline Normal saline/acetone	Soluble ^{99m}Tc impurities ^{99m}Tc hydrolyzed reduced
^{99m}Tc Cardiolite (Two strips)	ITLC-SG ITLC-SG	Normal saline Acetone	Free ^{99m}Tc pertechnetate ^{99m}Tc hydrolyzed reduced
^{99m}Tc Cardiolite (Single strip)	Whatman 31ET	Ethyl acetate	Free ^{99m}Tc pertechnetate ^{99m}Tc hydrolyzed reduced ^{99m}Tc polar impurities

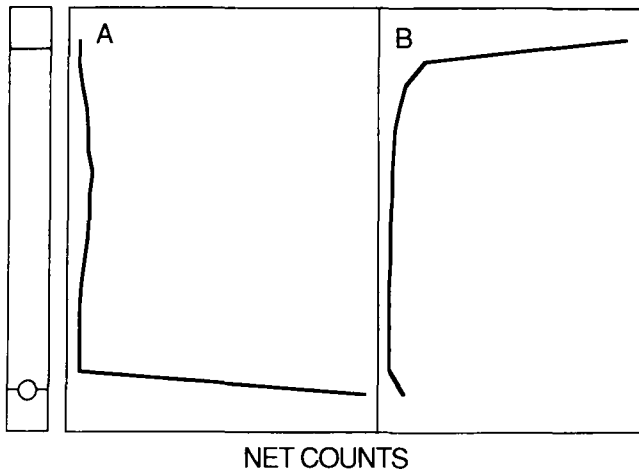


FIG. 3. Chromatography strip activity distribution of (A) ^{99m}Tc pertechnetate and (B) ^{99m}Tc exametazime in a chromatography system consisting of Whatman 17 with ethyl acetate.

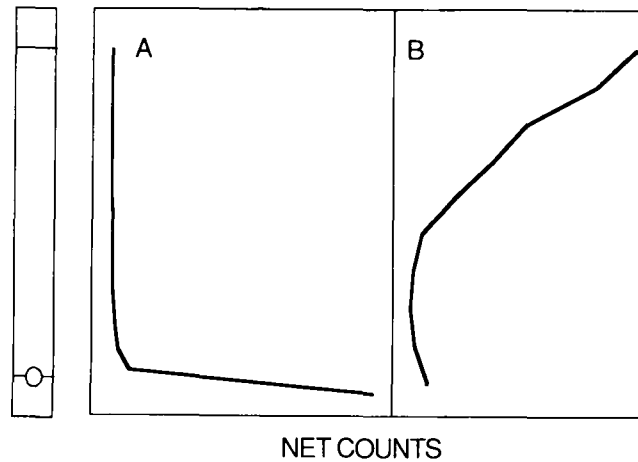


FIG. 5. Chromatography strip activity distribution of (A) ^{99m}Tc pertechnetate and (B) ^{99m}Tc sestamibi in a chromatography system consisting of Whatman 31ET with ethyl acetate.

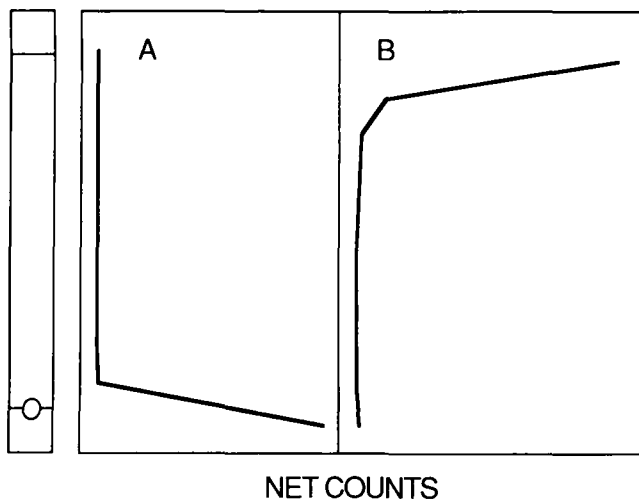


FIG. 4. Chromatography strip activity distribution of ^{99m}Tc teboroxime in chromatography system consisting of (A) Whatman 31ET with normal saline and (B) Whatman 31ET with acetone/normal saline (1:1).

manufactured by E. I. du Pont de Nemours, Billerica, MA. The recommended manufacturer's chromatographic quality control procedure (19) utilizes aluminum oxide plated thin layer chromatography plates. In addition, the manufacturer recommends a 15-min radiopharmaceutical spot drying time prior to strip development. Due to these factors, the time needed to perform radiochemical purity evaluations of ^{99m}Tc sestamibi, following the manufacturer's stated procedure is exceedingly long (30 min). As a result, a number of more rapid miniaturized chromatography procedures have been proposed and are listed in Table 4. The two-strip method (15), utilizes Gelman ITLC-SG (Gelman Instruments, Ann Arbor, MI) strips with normal saline and acetone as the respective solvents. With this system, free ^{99m}Tc pertechnetate and hydrolyzed reduced ^{99m}Tc are separated from ^{99m}Tc sestamibi. However, other radiochemical impurities may not be

identified with this chromatography system. Our laboratory is currently investigating a single-strip chromatography system that uses Whatman 31ET and ethyl acetate. A typical chromatography strip activity distribution of ^{99m}Tc sestamibi on Whatman 31ET strips is shown in Figure 5. With our system, free ^{99m}Tc pertechnetate and hydrolyzed reduced ^{99m}Tc remain at the origin, while ^{99m}Tc sestamibi migrates close to the solvent front—with some streaking. Due to the streaking, the cut line is located at an Rf value of 0.2–0.25. Possible polar radiochemical impurities would also remain at the origin.

Technetium-99m Mertiatide (TechneScan MAG3[™]). TechneScan MAG3 is manufactured by Mallinckrodt Medical Inc., St. Louis, MO. Quality control for ^{99m}Tc mertiatide preparations involves a technique known as solid phase extraction (20). With this technique, the radiolabeled sample is applied to a solid adsorbent, a Waters Sep-Pak C18 cartridge (Millipore Corp., Milford, MA), and various radiochemical components are selectively eluted in a step-wise manner using appropriate solvents, including 0.001 N HCl and ethanol/saline (1:1). The technique is outlined in the manufacturer's product insert and is fairly rapid and easy to use. However, possible technical errors can occur. Following radiopharmaceutical loading, the cartridge must be eluted slowly with ethanol/saline. If not eluted slowly, ^{99m}Tc mertiatide will remain on the cartridge resulting in false estimates of radiochemical purity. A number of attempts have been made to develop rapid thin layer or paper chromatography procedures to evaluate the radiochemical purity of ^{99m}Tc mertiatide. However, the attempts have not been successful.

CONCLUSIONS

Quality control of radiopharmaceuticals is an important part of the overall quality control procedures performed in the nuclear medicine department. For many of the newer radiopharmaceuticals, including ^{99m}Tc radiopharmaceuticals that are formulated within the nuclear medicine department, radiochemical purity assessment is mandatory prior to patient

injection. With these preparations, if certain levels of purity are not obtained, the radiopharmaceutical preparation cannot be clinically utilized.

This article was written to allow the nuclear medicine technologist or radiopharmacist to use rapid, yet accurate chromatography systems to evaluate the radiochemical purity of radiopharmaceuticals in a short time period. Some of the chromatography systems are modifications of the manufacturers' recommended systems. Others are newly developed systems which have been extensively tested in laboratories. The simplicity of these chromatography systems should encourage the nuclear medicine community to expand and improve its program of radiopharmaceutical quality control.

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