
Rapid Determination of the Radiochemical Purity of ^{99m}Tc -Antimony Trisulfide Colloid Prepared by Standard and Alternative Heating Methods

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The purpose of this study was to validate a rapid quality control method for the lymphoscintigraphic tracer ^{99m}Tc -antimony trisulfide colloid (^{99m}Tc -ATC). **Methods:** ATC was labeled with ^{99m}Tc according to the manufacturer's instructions as well as by alternative heating conditions designed to provide a range of percentages of radiochemical purity (RCP): the tracer was prepared in a dry block heater with heating cavities of different sizes, the temperature of the heating block was varied from 70°C to 115°C, or the duration of heating was varied from 15 to 35 min. Anion-exchange minicolumns were trialed to separate any ^{99m}Tc -pertechnetate impurity from ^{99m}Tc -ATC with physiologic saline as the eluent. Quality control results were compared with the results from the manufacturer's recommended method, which uses an instant thin-layer chromatography (ITLC) strip with saline as the migrating solution. **Results:** The quality control results obtained with a cartridge method in 2–3 min compared favorably with those obtained with the ITLC method with saline when the tracer was prepared by heating at 115°C in a dry block heater for 35 min (RCPs, 99.4% \pm 0.3% [mean \pm SD] and 99.2% \pm 0.3%, respectively; $n = 25$). The cartridge and ITLC quality control results also were in excellent agreement (correlation coefficient, 0.99) over a range of RCPs (80%–100%). An alternative anion-exchange cartridge that was tested in this study was not suitable for assaying the RCP of ^{99m}Tc -ATC because of the complete retention of ^{99m}Tc -pertechnetate on the sorbent. **Conclusion:** Compared with the established ITLC method, the cartridge quality control method tested in this study is rapid and provides a reliable assessment of the RCP of ^{99m}Tc -ATC. For the preparation of ^{99m}Tc -ATC, a dry block heater can be successfully substituted for a boiling water bath and is recommended for heating at high altitudes.

Key Words: antimony trisulfide colloid; radiocolloid; quality control; radiochemical purity; cartridge

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Radiocolloids are routinely used for lymphatic mapping and localization of sentinel lymph nodes for biopsy (1,2) in

the staging of patients with melanoma (3), breast (4), and other (5–9) cancers. In Australia and New Zealand, ^{99m}Tc -antimony trisulfide colloid (^{99m}Tc -ATC) is currently the lymphoscintigraphic tracer of choice because the radiocolloid has a nearly optimal particle size of approximately 5–20 nm (10). The uptake of radiocolloids from the interstitial space into the lymphatic lumen occurs through small gaps that exist between the overlapping endothelial cells comprising the lymphatic capillary walls. Previous studies demonstrated that particles smaller than 50 nm show the most rapid migration from the interstitial injection site and maximal permeation through lymphatic vessels (11), properties that facilitate early and reliable visualization of sentinel lymph nodes and lymphatic vessels (2). ^{99m}Tc -ATC particles preferentially migrate into the lymphatic system rather than the blood capillaries and are retained at high levels in lymph nodes, allowing intraoperative sentinel lymph node localization by use of a γ -probe for up to 24 h after injection (1–3).

The ^{99m}Tc -ATC solution is prepared by heating a mixture of acidified ATC and ^{99m}Tc -pertechnetate at 100°C for 30 min (12). The solution is cooled, buffered, and filtered before quality control analysis and administration to patients. The gold standard assay of percentages of radiochemical purity (RCP) uses ascending instant thin-layer chromatography (ITLC) and a 0.9% sodium chloride solution (saline) as the migrating solution (12). This quality control analysis can take 10–15 min to complete. In a busy dose preparation area, there is a need to determine the labeling efficiency of radiopharmaceuticals quickly and reliably by use of readily available apparatus and consumables. Chromatographic minicolumns have facilitated rapid and reliable quality control analyses of ^{99m}Tc -exametazime (13), ^{99m}Tc -bicisate (14), ^{99m}Tc -tetrofosmin (15), ^{99m}Tc -sestamibi (16), ^{99m}Tc -mertiatide (17), and ^{99m}Tc -aprotinin (18). The aim of this study was to validate a rapid cartridge quality control method for ^{99m}Tc -ATC over a range of RCPs.

MATERIALS AND METHODS

General

^{99m}Tc colloidal antimony sulfide injection was prepared with a commercial kit (Lymph-Flo; RAH Radiopharmacy), the contents of

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which were described previously (12). Sodium ^{99m}Tc -pertechnetate was obtained from the daily elution of a ^{99}Mo - ^{99m}Tc generator (Gentech; Australian Radioisotopes). All preparations were made in triplicate unless stated otherwise. Results are presented as mean \pm SD. Linear regression analysis was performed to compare the results determined with the cartridge method and the ITLC method.

Preparation of ^{99m}Tc -ATC with Boiling Water Bath

^{99m}Tc -ATC was prepared according to the manufacturer's instructions. In brief, after the addition of ^{99m}Tc -pertechnetate (900–1,000 MBq) in saline (1.0 mL) and hydrochloric acid (0.1 mL, 1.0 mol/L) to the antimony trisulfide vial (1.0 mL), the breather needle was removed and the vial was submerged in boiling water. After 30 min, the vial was removed from the bath and allowed to cool for 5 min in an aluminum block; this step was followed by the addition of phosphate buffer (0.5 mL) and subsequent filtration (0.2 μm) into an empty vial.

In a second group of preparations, the procedure was repeated as described previously, except that the duration of heating in the boiling water bath was increased to 35 min.

Alternative Preparation of ^{99m}Tc -ATC with Dry Block Heater

^{99m}Tc -ATC was prepared as described previously, except that a temperature-controlled dry block heater (DBH20D; Ratek Instruments) was used to heat the reaction vial at different temperatures and for variable durations. The digital temperature readout was validated with a thermometer. Two different custom aluminum heating blocks (Ratek Instruments) were inserted into the heating apparatus. The 2 blocks differed in the diameter and depth of the 6 holes that could accommodate the 20-mm-diameter reaction vials. One block featured 21-mm-diameter holes to a depth of 40 mm, and the other block had 26-mm-diameter holes to a depth of 43 mm.

For a direct comparison with the manufacturer's recommended method, ATC was mixed with 900–1,000 MBq of ^{99m}Tc -pertechnetate and acid and then heated in the 21-mm dry block at 100°C or 115°C for 30 min.

For all further heating block experiments, 200–400 MBq of ^{99m}Tc -pertechnetate in saline (1.0 mL) were used. The 26-mm heating block was trialled by heating at 115°C for 30 or 35 min. In a second group of experiments, the radiolabeling conditions were kept constant by heating in the dry block (21-mm) at 115°C for 35 min ($n = 25$). In a third set of experiments, the heating temperature was kept constant at 115°C while the heating duration was varied from 15 to 35 min. In a fourth group of experiments, the heating duration was kept constant at 35 min while the heating temperature was varied from 70°C to 100°C.

Quality Control Analysis

The quality control analysis of the various preparations took place after filtration of the colloid. Each preparation was analyzed for the RCP of ^{99m}Tc -ATC by use of ascending ITLC with a silica gel-impregnated glass fiber strip (ITLC-SG; 1×16 cm; Gelman Sciences) and 0.9% saline as the solvent or by use of a strong anion-exchange minicolumn (Amprep SAX; 100 mg, 1 mL; Amersham Biosciences) with saline as the eluent. The 2 quality control tests were performed concurrently.

Radiochemical Purity of ^{99m}Tc -ATC Determined by ITLC

Each ITLC strip was marked at 2 cm from one end of the strip for the origin and every 1 cm until the solvent front was marked at 10 cm

from the origin. A small sample from the kit was diluted with saline (to 0.1 mL), and a single drop was spotted onto the ITLC strip at the origin. The strip was developed in saline and then cut into 1-cm segments, which were placed in a γ -counter (Packard Auto- γ -5650; Hewlett-Packard) for counting over a ^{99m}Tc window (70–210 keV). The percentage of ^{99m}Tc -pertechnetate was calculated to be the combined activity of sections with $R_f \geq 0.7$ divided by the total activity, and the percentage of ^{99m}Tc -ATC was calculated to be the percentage of sections with $R_f < 0.7$.

Radiochemical Purity of ^{99m}Tc -ATC Determined by Anion-Exchange Minicolumn Chromatography

The SAX minicolumn was rinsed with saline (2 mL) before use. A sample (<0.05 mL, 5–15 MBq) of the filtered solution was added dropwise onto the sorbent bed. The cartridge was eluted with saline (4 mL) at a flow rate of 2 or 3 drops per second, followed by an air space (1–2 mL), into a collection vial. The contents of the cartridge and the vial were counted separately in a validated counting unit (Atomlab 100⁺ dose calibrator; Biodex Medical Systems). All sample counts were background corrected.

Elution Profile of ^{99m}Tc -Pertechnetate

^{99m}Tc -pertechnetate (~ 10 MBq) in saline (0.05 mL) was loaded onto the sorbent bed of 2 different anion-exchange cartridges, the Amprep SAX cartridge described earlier and the Oasis MAX cartridge (30 mg, 1 mL; Waters Corp.). The cartridges were eluted with saline (4 mL) at a flow rate of 3 drops per second into empty glass vials. The contents of the cartridges and collection vials were counted in the dose calibrator as described previously.

RESULTS

Preliminary Evaluation of Anion-Exchange Cartridge Methods

^{99m}Tc -ATC bound to the Amprep SAX sorbent, as observed visually by an orange band in the sorbent and a clear eluate in the collection vial. The time taken to perform the Amprep SAX cartridge method was 2–3 min. When ^{99m}Tc -pertechnetate was added to the Amprep SAX cartridge, it eluted off the cartridge with 4 mL of saline such that $99.8\% \pm 0.2\%$ of the activity was found in the eluate. Conversely, ^{99m}Tc -pertechnetate was strongly retained by the Oasis MAX cartridge when it was eluted with 4 mL of saline such that 100% of the activity was found on the cartridge.

Accuracy of Amprep SAX Cartridge Method

The RCPs of ^{99m}Tc -ATC samples heated for 30 or 35 min in a boiling water bath or dry block heater are shown in Table 1. When the samples were heated in a boiling water bath for 30 min, the RCP determined by the SAX cartridge method was $98.0\% \pm 0.8\%$, and that determined by the ITLC method was $97.6\% \pm 0.9\%$, with a mean absolute difference ($|\Delta|$) of $0.4\% \pm 0.1\%$. Results comparable to those obtained with the boiling water bath preparation were obtained for the 21- and 26-mm heating block experiments after 30 min. When the samples were heated in the 21-mm dry block at 100°C, the RCPs determined by the SAX method and the ITLC method were 97.3% and 96.9%,

TABLE 1
RCPs of ^{99m}Tc -ATC Determined by ITLC and SAX Methods ($n = 3$)

Method	RCP (%)					
	30 min			35 min		
	ITLC	SAX	$ \Delta $	ITLC	SAX	$ \Delta $
Water bath at 100°C	97.6 ± 0.9	98.0 ± 0.8	0.4 ± 0.1	98.1 ± 0.5	98.2 ± 0.5	0.1 ± 0.0
Dry block at 21-mm and 100°C	96.9 ± 0.4	97.3 ± 0.4	0.4 ± 0.0	97.6 ± 0.3	97.9 ± 0.3	0.3 ± 0.0
Dry block at 21-mm and 115°C	99.1 ± 0.2	99.3 ± 0.1	0.2 ± 0.1	99.3 ± 0.4	99.3 ± 0.3	0.2 ± 0.1
Dry block at 26-mm and 115°C	98.0 ± 0.4	98.3 ± 0.4	0.4 ± 0.1	98.5 ± 0.6	98.4 ± 0.2	0.4 ± 0.1

respectively; these values increased approximately 2% to 99.3% for the SAX method and 99.1% for the ITLC method when the samples were heated at 115°C. The effect of using the slightly larger 26-mm dry block heating cavity was minimal when the samples were heated at 115°C for 30 min, with the SAX and ITLC methods recording RCPs of 98.3% and 98.0%, respectively. The $|\Delta|$ between the SAX and ITLC results was very low for triplicate experiments, ranging from 0.1% to 0.5%.

For the large series of ^{99m}Tc -ATC samples prepared with a dry block heater (21-mm) set at 115°C for 35 min ($n = 25$), the mean quality control results (RCPs) obtained with the SAX and ITLC methods were 99.4% ± 0.3% and 99.2% ± 0.3%, respectively. The results obtained with the 2 methods for the individual samples differed by 0.0%–1.0%, with a $|\Delta|$ of 0.3% ± 0.2%.

The quality control results obtained with the SAX and ITLC methods for the samples heated in the dry block heater at 115°C for 15–35 min are shown in Figure 1. After 15 min, the RCP of ^{99m}Tc -ATC determined by the cartridge method was 91.9% ± 0.4%; that determined by the ITLC method was 91.5% ± 0.5%. The RCPs at the 20-, 25-, 30-, and 35-min time points were 96.0% ± 0.5%, 97.9% ± 0.2%, 99.3% ± 0.1%, and 99.3% ± 0.3%, respectively, as determined by the cartridge method and 96.0% ± 0.5%, 97.7% ± 0.5%, 99.2% ± 0.5%, and 99.3% ± 0.4%, respectively, as determined by the ITLC method. The $|\Delta|$

between the 2 methods for the various time points ranged from 0.1% to 0.4%.

The quality control results obtained with the SAX and ITLC methods for the samples heated in the dry block heater for 35 min at 70°C–115°C are shown in Figure 2. When determined by the SAX cartridge method, the RCP rose from 85.0% ± 1.5% when the samples were prepared at 70°C to 90.1% ± 0.4%, 95.7% ± 0.3%, 97.4% ± 1.1%, 97.9% ± 0.3%, and 99.3% ± 0.3% when the samples were heated at 80°C, 90°C, 95°C, 100°C, and 115°C, respectively. Equivalent results were obtained with the ITLC method, which yielded RCPs of 83.7% ± 1.3%, 90.2% ± 0.3%, 95.1% ± 0.2%, 97.5% ± 1.0%, 97.6% ± 0.3%, and 99.3% ± 0.4% for the 70°C–115°C reactions, respectively. The $|\Delta|$ between the 2 methods for the various temperatures ranged from 0.2% to 1.3%.

A plot of the RCPs obtained by the SAX method versus the RCPs obtained by the ITLC method for all samples ($n = 55$) is shown in Figure 3. The linear regression equation was as follows: % SAX = (0.95 × % ITLC) + 5.3%; the correlation coefficient was 0.99.

Precision of SAX Method

The precision of the SAX method was determined by testing a single vial of ^{99m}Tc -ATC prepared by heating in a dry block heater for 35 min at 115°C. Five samples were assayed and yielded an RCP of 99.1% ± 0.1%.

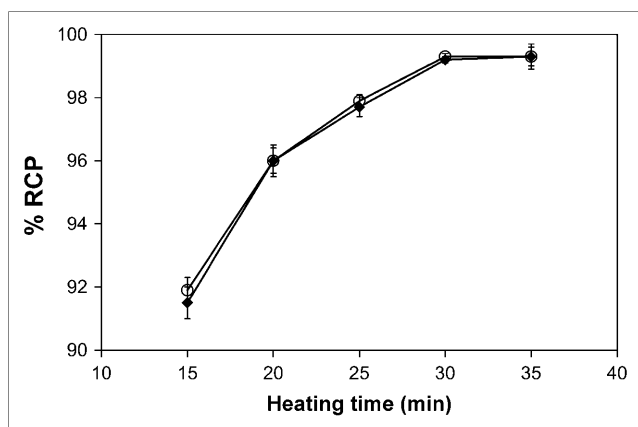


FIGURE 1. RCPs, determined by SAX (○) and ITLC (◆) quality control methods, of ^{99m}Tc -ATC samples ($n = 3$) prepared with dry block heater set at 115°C for various durations.

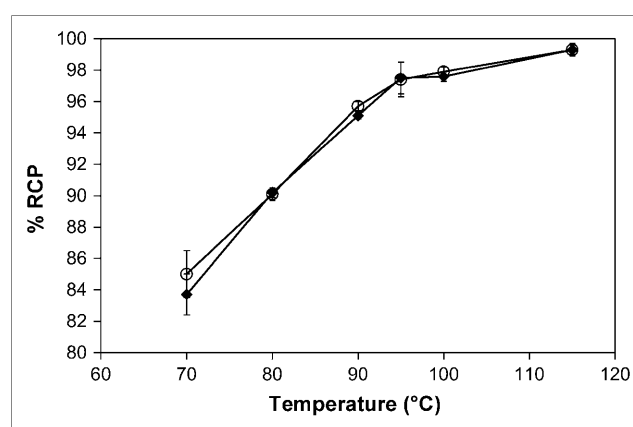


FIGURE 2. RCPs, determined by SAX (○) and ITLC (◆) quality control methods, of ^{99m}Tc -ATC samples ($n = 3$) prepared with dry block heater set at various temperatures for set time of 35 min.

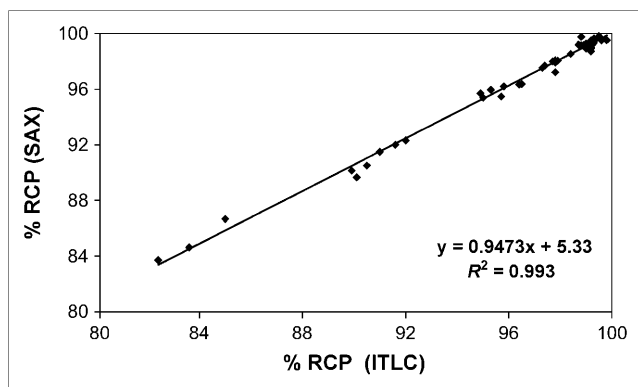


FIGURE 3. Plot of RCPs determined by SAX method vs. RCPs determined by ITLC method for each ^{99m}Tc -ATC sample tested in this study.

DISCUSSION

^{99m}Tc -ATC is formed in situ at a high RCP after the product is prepared under standard conditions of heating ^{99m}Tc -pertechnetate with an acidic colloidal dispersion in a boiling water bath for 30 min. The tracer is suitable for patient injection when the RCPs are greater than 95%, according to the manufacturer's guidelines. The recommended quality control method uses ITLC-SG with saline as the solvent and requires a sensitive counting apparatus and 10–15 min to complete. The goal of this study was to validate a quality control technique based on the use of a chromatographic minicolumn similar to those used for several other ^{99m}Tc -labeled radiopharmaceuticals (13–18). For a cartridge method to be successful, a clear chromatographic separation of the ^{99m}Tc impurities from the ^{99m}Tc product needs to take place. A strong anion-exchange cartridge was hypothesized to be appropriate for quality control trials of ^{99m}Tc -ATC because the colloidal particles have a negatively charged surface (12,19) and were expected to bind strongly to the positively charged sorbent. However, the success of the hypothesized method depended on whether the saline solvent completely eluted the unreacted ^{99m}Tc -pertechnetate anions ($^{99m}\text{TcO}_4^-$) from the cartridge. In this study, ^{99m}Tc -pertechnetate was found to be completely eluted from the Amprep SAX cartridge when 4 mL of saline was used but remained bound to the Oasis MAX cartridge, rendering the latter unsuitable for further ^{99m}Tc -ATC quality control validation experiments. The reason for the difference in ^{99m}Tc -pertechnetate-binding affinity between the Amprep SAX and Oasis MAX cartridges is not clear. Both cartridges have strong anion-exchange properties because of the presence of very similar quaternary amine functionalities but differ in the chemical compositions of the polymeric backbones of the sorbents. The Amprep SAX cartridge features a silica-based polymeric backbone that is chemically treated to minimize any interfering effects of the silica substrate on the primary anion-exchange interactions. The Oasis MAX cartridge features as a backbone a poly(divinylbenzene-co-*N*-vinylpyrrolidone) copolymer that is specifically de-

signed to facilitate the retention of organic anions. It may be this difference in backbone structure that causes the ^{99m}Tc -pertechnetate anions to be more strongly bound to the Oasis MAX cartridge.

When a large series of ^{99m}Tc -ATC samples ($n = 25$) was prepared under optimal conditions, the mean SAX and ITLC quality control results were nearly identical, differing by only 0.2%. As the level of ^{99m}Tc -pertechnetate was less than 1%, further experiments were designed to investigate the accuracy of the SAX method over a range of RCPs at which the levels of impurities were much higher. The variables used to achieve this goal were the heating mechanism (Table 1), duration (Fig. 1), and temperature (Fig. 2). The correlation between the 2 quality control methods for the complete dataset was excellent over the RCP range of 80%–100% (Fig. 3), with the $|\Delta|$ between the results of the 2 tests being $0.3\% \pm 0.3\%$ ($n = 55$). The greatest divergence between the SAX and ITLC results was only 1.3%, observed when ^{99m}Tc -ATC was prepared at 70°C (Fig. 2).

The boiling water bath and a dry block heater with 21- or 26-mm-diameter heating cavities were each successfully used to prepare ^{99m}Tc -ATC at RCPs of greater than 95% (range, 97%–99%). Both heating blocks were trialed at 115°C, at which the 21-mm cavity increased the RCP by approximately 1% over that obtained with the 26-mm cavity or the boiling water bath.

When the temperature of the 21-mm dry block heater was decreased, the RCPs were greater than the 95% cutoff at 100°C and 95°C, approximately equal to 95% at 90°C, but well below 95% at both 80°C and 70°C. The data obtained with the dry block heater at reduced temperatures also may provide an approximate guide to results that may be attained with the boiling water bath protocol at high altitudes. At a 1,500-m elevation (e.g., Denver at 1,610 m and Johannesburg at 1,700 m), water boils at approximately 95°C (20); this property would have a minor effect on the RCP of ^{99m}Tc -ATC when prepared according to the manufacturer's heating protocol. In some very-high-altitude cities, in which water boils at close to 90°C (e.g., Bogotá at 2,640 m, Quito at 2,810 m, and La Paz at 3,660 m), ^{99m}Tc -ATC would be best prepared with a dry block heater set at 100°C–115°C. According to the 90°C results obtained in this study, following the manufacturer's boiling water bath protocol at an elevation of about 3,000 m would yield ^{99m}Tc -ATC with an RCP close to the 95% cutoff for patient administration.

Reduced heating time was the other variable that was introduced into the study to generate a range of RCPs. After the samples were heated in a dry block heater at 115°C for 15 min, the RCP already had exceeded 90%, but it took another 5 min of heating to exceed the 95% cutoff (Fig. 2). The RCP continued to rise to a maximum of 99% at 30 min.

CONCLUSION

The Amprep SAX-saline quality control method was shown to provide rapid and reliable determination of the

RCP of ^{99m}Tc -ATC when compared with the recommended ITLC method. The straightforward cartridge method saves time and uses common consumables as well as a routine dose calibrator, features that will assist a busy radiopharmacy or a small nuclear medicine practice that does not have the sensitive counting apparatus required for ITLC. The Oasis MAX anion-exchange cartridge was not useful for this application because of the strong retention of the anionic impurity ^{99m}Tc -pertechnetate. For the preparation of ^{99m}Tc -ATC, a dry block heater can be successfully used to perform the heating operation and will ensure that the RCP exceeds 95% at high altitudes.

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