Experiment Notebook Format

Experiment Notebooks will be distributed during the first weeks of the second Quarter.

Quarter 2 experiments to be completed:

Lab A: Count Rate, Time and Distance
Lab B: Determine the chemical purity of Tc^{99m} MDP and Tc^{99m} MAA
Lab C: Calculate Actual and Theoretical dead time
Lab D: Spatial Resolution

Format

Typewritten report must include:

1) Title Page with Name and Date(s) of Experiment
2) Table of Contents
3) Experiment Body to include:
   - Purpose
   - Description of Procedure
   - Materials/Equipment Used
   - Technique
   - Data
   - Graphs or Films (Most systems allow you to right-click on an image to save a digital copy to disk)
   - Discussion of Data
4) Conclusion

These experiments are designed for independent study but should always involve appropriate supervision from clinical support staff. Experiments can be completed at any institution. Labs are to be done as time permits and must not interfere with the department's responsibility to patient care. These experiments are time consuming so plan accordingly.

Experiment Notebook
Quarter 2 – Lab B
Chromatography (Chemical Purity Determination)

References: http://nuclearpharmacy.uams.edu/general.htm (check out the movie)

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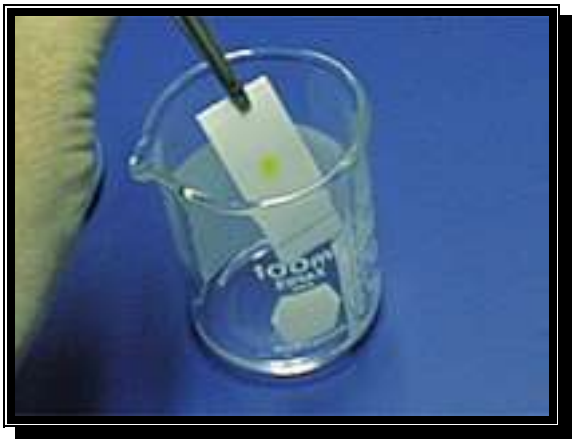


Thin Layer Chromatography

These experiments are to be completed under the supervision of a licensed technologist within the affiliate's nuclear medicine department. Experiments shall not interfere with clinical duties.

BACKGROUND

The radiochemical purity of a radiopharmaceutical is the fraction of the total radioactivity in the desired chemical form in the radiopharmaceutical. Radiochemical impurities arise from decomposition due to the action of the solvent, change in temperature of pH, light and radiolysis. Examples of radiochemical impurities are free $^{99m}TcO_4^-$ and hydrolyzed ^{99m}Tc in many Technetium-labeled complexes. The presence of radiochemical impurities in a radiopharmaceutical results in poor-quality images due to poor localization in the organ of interest and the high background from the surrounding tissues.

INTRODUCTION

A number of analytical methods can be used to detect and determine the radiochemical impurities in a given radiopharmaceutical. The purpose of this experiment is to identify the radiochemical purity of given radiopharmaceuticals using instant thin layer chromatography (ITLC).

In chromatography, a small amount of the radiopharmaceutical is spotted at a predetermined are on the strip (referred to as the origin). The strip is then placed in a vial that contains a small amount of the appropriate solvent. The strip is placed in such a way that the spotted end is dipped into the solvent but the spot remains above the spotted level. When the solvent front moves to a desired distance, the strip is removed from the vial and counted. If a well counter is used, the strip must be divided at a designated spot and both halves (origin and solvent front) are counted. If a strip reader is used, the strip remains in one piece and is automatically counted.

Chromatography involves the separation of a chemical mixture (in this case, a radiopharmaceutical) into its components along a stationary phase (the strip) as a result of different velocities in the mobile phase (migrating solvent). The presence of a component is determined by the location of its radioactivity on the strip. Given below are the migratory characteristics of commonly used radiopharmaceuticals.

Group A: **Sulfur colloid, Albumin Colloid, MAA**
These preparation are insoluble in both acetone and saline and do not move from the origin. Thus, the amount of oxidized $^{99m}TcO_4^-$ can be determined because it migrates with the solvent front. Use saline as the solvent and strips designated for use in saline. Ask your local pharmacy if they can send a few SG/Whatman strips along with that day's order.

Group B: **MDP, DTPA, PYP, Glucoheptonate**
These preparations are soluble in saline, but are insoluble in acetone. The amount of oxidized $^{99m}TcO_4^-$ can be determined using acetone because the radiopharmaceutical remains at the origin and any free $^{99m}TcO_4^-$ moves with the solvent front. Saline is used to determine the amount of hydrolyzed (TcO_2) product. The hydrolyzed product remains at the origin while the radiopharmaceutical that is soluble in saline moves with the solvent front.

MATERIALS

1. Well counter
2. ITLC Silica-Gel (SG) strips (~8cm in length).
3. Whatman 3mm strips (~8cm in length).
4. ITLC vials
5. Dispensing syringe with small (e.g. 25-guage) needle.
6. 100% Acetone (Nail polish remover - available at Walgreen's or Long's)
7. Saline (0.9%)

PROCEDURE

Generator Elution (Don't Repeat: We performed during the 1st week of the program)

1. Elute a 99-Mo/99mTc generator by concurrently inverting a 10cc saline vial and a shielded 10cc evacuated vial over the appropriate ports for 1 minute.
2. Ensure that Al^{3+} ion and Molybdenum breakthrough are with USP limits by spotting a sample on a colorimetric strip and then assay the full eluate in the dose calibrator using a "moly shield".
3. Spot a small drop of generator eluate at the origin of the appropriate ITLC strip (see figures 1 - 3). The spot must be tiny (~½ the width of the strip).
4. Compound one kit from each Group per manufacturer's specifications
5. Place strip in appropriate solvent as specified in Figure 1 and 2 (Solvent volume should be sufficient to allow liquid to migrate to the top of the ITLC strip without submerging the origin-spot. Hold with forceps taking care not to let the strip adhere to the sides of the test tube. This will cause the strip to become instantaneously saturated altering migration).
6. Remove the strip after the solvent front has migrated vertically almost to the top mark (approximately 45 seconds). Do not let the solvent migrate all the way to the top of the strip.
7. Using hemostats to grip the top of the strip. Cut at the designated site (See Figure 2). Place both halves in separate counting vials
8. Count each vial separately in the well counter for 10 seconds.

Data from the Generator we eluted in Week 1

Amount Eluted	96 mCi Tc99m eluted in 10 cc volume
Molybdenum Assay	0.14 µCi Mo-99
Aluminum Ion	<10 ppm
Top half of QC strip	Top counts = 3760408
Bottom half of QC strip	Bottom counts = 22358

9. To complete the following procedures, you can order new doses but it would be best if you use leftover doses from 'no-show' patients (especially if you're performing experiments at a non-VA clinic). Better yet, the small volume remaining in the hub of the needle from a patient dose probably will suffice for this experiment.

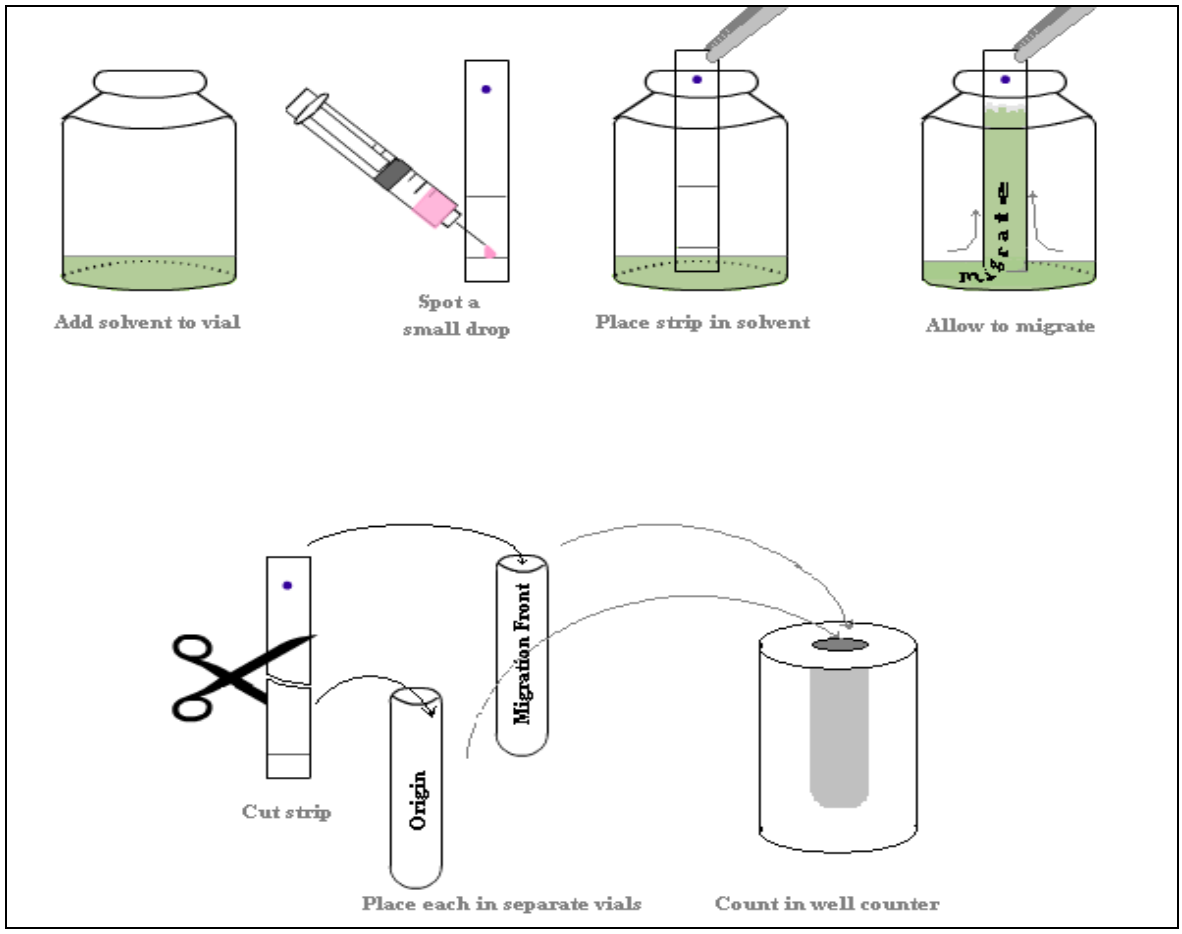
Group A Procedure (99mTc Sulfur Colloid or 99mTc MAA)

10. Spot a small drop of radiopharmaceutical A at the origin of the appropriate ITLC strip (see figures 1 – 3 on the following page).
11. Repeat steps 5 – 8 on the previous page using the solvent listed in fig 7.

Group B (99mTc MDP or 99mTc DTPA)

12. Spot a small drop of radiopharmaceutical B at the origin of both an SG and Whatman 3mm ITLC strip (see figures 5 - 7 on page 7).
13. Repeat steps 5 – 8 from the previous page using the solvents listed in fig 7.

Figure 1: Thin Layer Chromatography Basics



Common Mistakes:

- The spot must be very small. Try “brushing” a drop off the tip of the syringe onto the strip rather than attempting to push it out with the plunger of the syringe. Always use shielding and gloves (See figure 2).

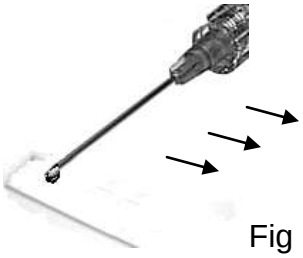


Fig 2: Brush off droplet onto origin of the QC strip (don’t squirt Tc99m or you risk creating a mess)

- Solvent volume must be sufficient for full migration but not so deep as to submerge the spot at the origin (see figure 3). Be sure to spot at 1cm above the bottom edge. Also make sure the spot is small (don’t saturate the bottom of the strip with Tc99m).

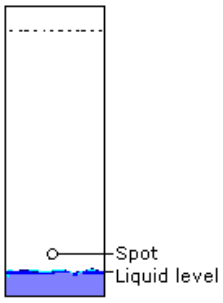


Fig 3

- Migration time must be long enough to allow the migration front to reach a level just below the top of the strip. If allowed to migrate too long, the solvent sample will reach the top and then rapidly drip back down to the origin skewing results. Migration time typically lasts for 30 seconds to no longer than 3 minutes.

- ITLC strip may stick against the side of the tube when wet (figure 4). This will instantaneously saturate the entire strip altering the normal migration. It is best to use forceps to hold the strip within the solvent and away from the walls of the vial for the 60 second (or so) migration period. You may also suspend the strip from a paperclip placed across the top of the beaker as seen in Figure 6 (next page).

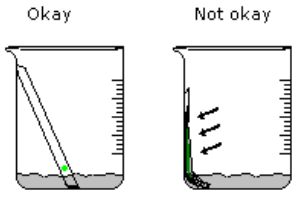


Fig 4

Figure 5 – USP XXIII Purity Procedure for Mo/Tc Generator Eluate

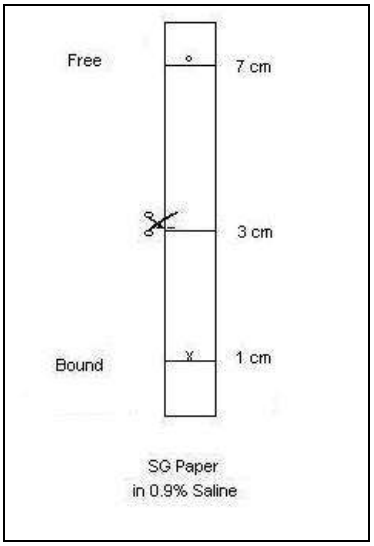


Figure 6

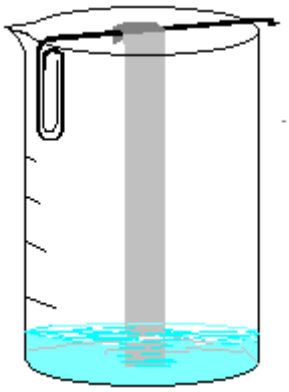
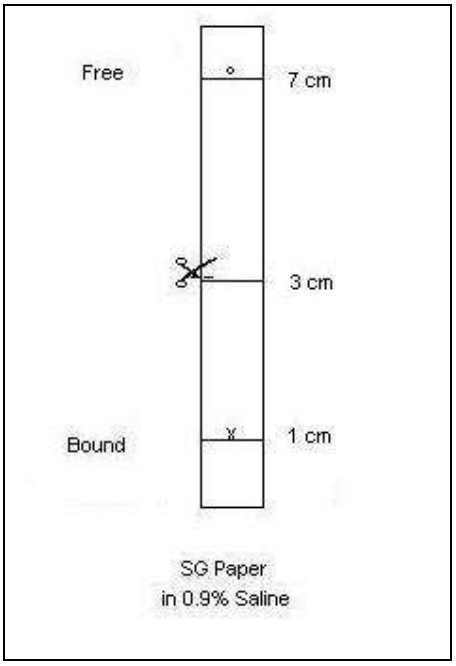
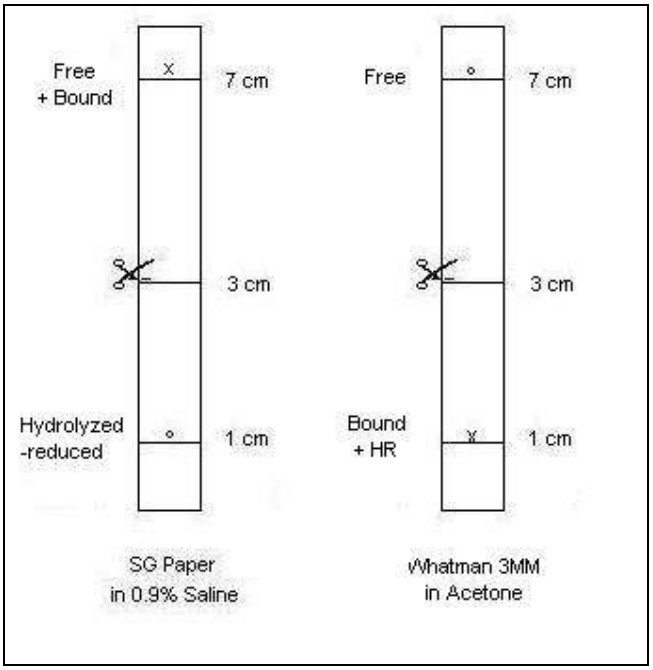


Figure 7 – USP XXIII Purity Procedure

Group A



Group B



DATA TREAMENT

As part of your data analysis, be sure to address the following questions:

1. List the USP XXIII limits for Al^{3+} ion and Molybdenum breakthrough of the generator eluate along with radiochemical purity limit for pertechentate.

2. List the USP minimum acceptable purities for each radiopharmaceutical from Group A and Group B.

3. Using the following formula, determine the percent $Na^{+}TcO_4^{-}$ for the generator data.

   % Free Tc-99m = top counts/(bottom counts + top counts) x 100

4. Using the following formulas, determine the percent tagged for the radiopharmaceutical from Group A:

   % Free ^{99m}Tc = (top counts)/(bottom counts + top counts) X 100
   % Tagged radiopharmaceutical = 100 - % free ^{99m}Tc

5. Is this radiopharmaceutical of acceptable quality? Explain what factors might lead to unacceptable purity results.

6. Using the following formulas, determine the percent tag for the radiopharmaceutical from Group B:

   Acetone: % free ^{99m}Tc = (top counts)/ (bottom counts + top counts) X 100
   Saline: % HR ^{99m}Tc = (bottom counts)/ (bottom counts + top counts) X 100
   % tagged radiopharmaceutical = 100- (% free ^{99m}Tc + % HR ^{99m}Tc)

7. Is this radiopharmaceutical of acceptable purity? Note if you get a failing result, it is likely not the result of a poorly manufactured kit. It would be best to repeat the experiment taking care to avoid the common mistakes described previously.

8. What is meant by hydrolyzed-reduced (HR) tech?

CONCLUSION

What lessons can be learned from this experiment? What are the potential consequence following administration of a poorly compounded kit?