Technical Artifacts in Chromatographic Analysis of Tc-99m Radiopharmaceuticals

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We investigated technical artifacts produced during chromatographic analysis of technetium radiopharmaceuticals. Such artifacts are, we found, caused by improper spotting and drying techniques; these in turn produce spuriously high impurities in Tc-99m complexes of DTPA, MDP, PPi, and GH. The ITLC-SG/acetone system produces considerable streaking of Tc-complex if the applied spot is large and not dried before development. This results in activity in the solvent front portion of the chromatographic strip indicating falsely high levels of pertechnetate impurity. Proper drying of the applied spot eliminates the artifact. The ITLC-SG/saline system yields falsely high, hydrolyzed-reduced technetium impurities if the spot is allowed to enter the solvent during development. Correct spot placement and size eliminate this problem. Strips that are allowed to dry in room air for several minutes may indicate considerable pertechnetate impurity on the chromatogram; yet this may not actually be present in the radiopharmaceutical vial. Drying spots rapidly with hot air or in a nitrogen atmosphere before development eliminates this problem.

There are numerous methods available to analyze technetium radiopharmaceuticals for radiochemical purity; however, instant thin layer chromatography (ITLC) is most frequently used because it is simple and rapid. Current ITLC methods are an outgrowth of systems used during the earlier days of Tc-99m radiopharmaceutical development. At that time, formulation methods centered around incorporation of technetium into various chemical forms. These methods generally did not yield quantitative labeling and, therefore, the free pertechnetate impurity needed to be removed prior to use. Purification was usually accomplished by the anion exchange or the gel chromatography system. Paper chromatography in 85% methanol was used to measure the extent of purification and to assess the purity of the finished product with time, since technetium compounds readily oxidize to regenerate pertechnetate.

In 1972 Eckelman and Richards (*1*), in an elegant study using anion exchange, gel, and paper chromatography, reported the presence of another radiochemical impurity in Tc-99m radiopharmaceuticals that they called hydrolyzed-reduced technetium. This impurity is a chemically-reduced form of technetium that is not complexed and forms a highly insoluble species, which exists in these preparations as a radiocolloid. Their report demonstrated that paper chromatography in saline solvent could identify three potential species of technetium radiopharmaceuticals—namely, Tc-99m complex, pertechnetate, and hydrolyzed-reduced technetium.

The introduction of many new kits for production of technetium-99m radiopharmaceuticals in nuclear medicine has created a need for rapid methods of chromatographic analysis. Since paper chromatography systems were slow and required nitrogen atmospheres to prevent oxidation, ITLC methods were developed. In 1973 Billinghurst (*2*) compared several methods of analyzing technetium compounds and reported that the ITLC-silica gel (SG) medium with saline and acetone solvents could rapidly and accurately identify technetium impurities in its radiopharmaceuticals. Subsequent to this, other reports appeared in the literature describing miniaturized systems that provided rapid analytical results with minimal expenditure of time (*3,4*). Most of the emphasis in all these reports was placed on solvent systems and support media with little attention directed toward fundamental techniques of spotting, drying, and solvent development, and the artifactual results that may be produced if improper technique is used. Careful technique is of paramount importance if miniaturized systems are used to analyze Tc-99m radiopharmaceuticals.

We found only one report (*5*) that dealt specifically with technical parameters associated with miniaturized chromatography systems. One important finding in

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this report was that significant errors are produced when chromatography strips are counted too closely to the scintillation detector. Additionally, spot size and time of development after spotting were also evaluated but only two radiopharmaceuticals were studied and the results obtained were somewhat inconclusive.

On several occasions we have received inquiries from technologists who reported inconsistent analytical results with chromatography. All of these reports noted high levels of either pertechnetate or hydrolyzed-reduced technetium in DTPA, glucoheptonate, or bone imaging agents. In most instances, improper radiochromatographic technique was discovered to be the problem.

We describe how artifacts are easily produced during chromatographic procedures and how they can be eliminated.

Materials and Methods

In all experiments the support medium was ITLC-SG (Gelman Instrument Co.). Solvents used were 0.9% sodium chloride injection USP (saline) and analytical grade acetone. Chromatographic strips were prepared in either 1 × 5 cm or 1 × 10 cm size and dried in a 105°C oven for 15 min before use. Technetium-99m complexes of sodium pentetate (DTPA/Medi-Physics Inc.), sodium gluceptate (GH/NEN), sodium medronate (MDP/NEN and E.R. Squibb), sodium pyrophosphate (PPi/Mallinckrodt Inc.), and sodium pertechnetate were prepared to contain 35 mCi in 4-ml final volume. Scintillation vials were used to contain 1 ml of acetone solvent. Test tubes (13 × 100 mm ID) were used to contain the saline solvent. Microliter pipettes of various capacities were used for spotting.

Chromatographic Technique: The general method used is illustrated in Fig. 1. Strips of 1 × 5 cm are routinely prepared by placing a pencil line 1 cm from the bottom of each strip marking the origin of spot placement. A pencil line at 2.5 cm marks where the strip is cut before radioassay and a spot of permanent ink (acetone system) or washable ink (saline system) is placed 3 to 5 mm from the top to mark the solvent front. This ink will migrate when the solvent reaches the top indicating that development is complete. Ideally, a 1- to 3-μl spot of the Tc-99m radiopharmaceutical is placed at the origin. A wet spot chromatogram is developed in saline but the spot must be dried before development in acetone. Strips are placed into the solvent so that the entire spot remains above the solvent level. Following development, strips are removed and dried and the origin and solvent front portions are independently radioassayed to determine the percent pertechnetate, hydrolyzed-reduced technetium, and technetium complex. This system is used for technetium complexes of DTPA, MDP, HEDP, PPi, and GH.

Wet versus Dry Spots: To determine the effect of developing a chromatogram with a wet or dry spot in

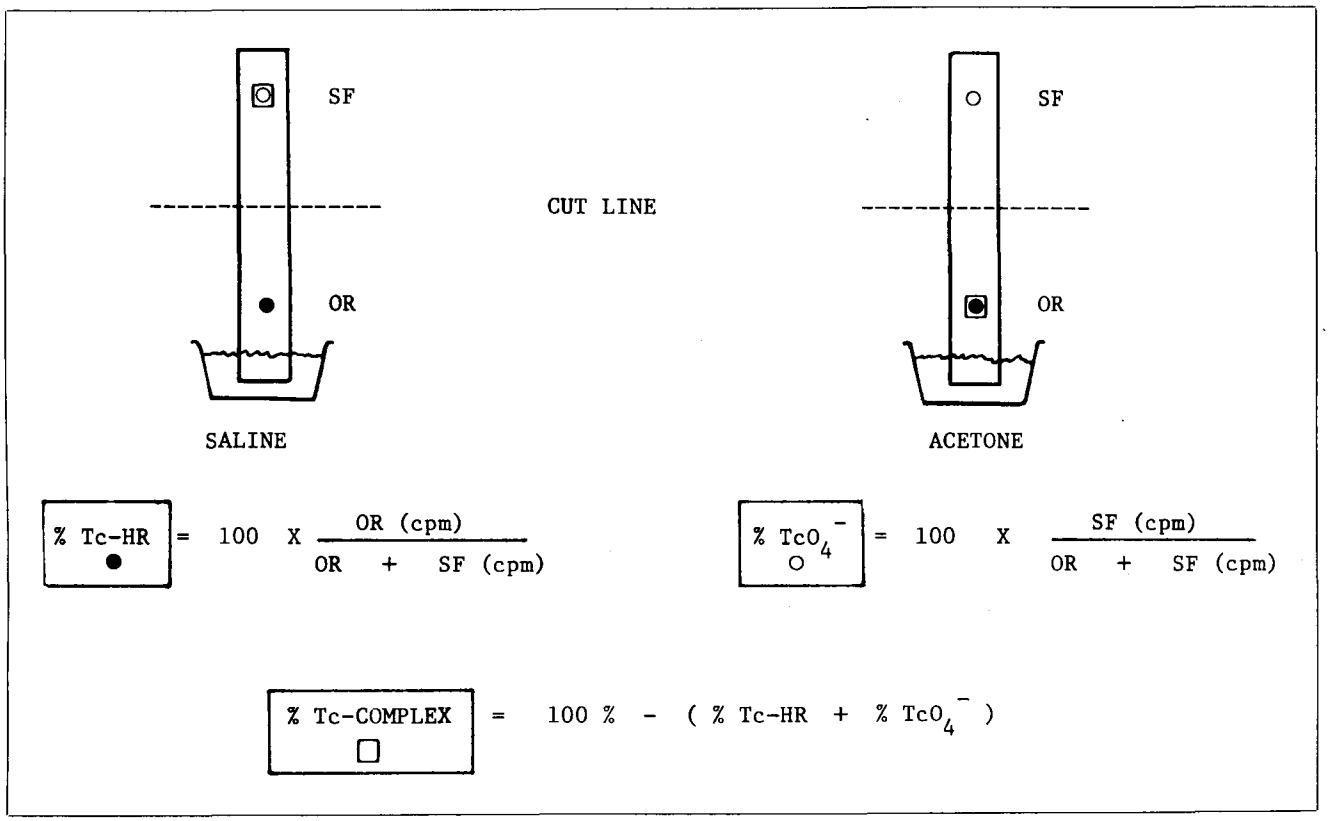

FIG. 1. ITLC-SG chromatography system for Tc-99m complexes of DTPA, MDP, GH, and PPi. OR indicates origin and SF indicates solvent front.

acetone, 10 strips (1 × 5 cm) were prepared for each technetium complex; 3 μl was spotted at the origin of each strip. Five strips were dried immediately for 20 sec with a hot air blower and developed. The remaining five strips were developed in like manner but with wet spots. Following development, strips were dried, cut, and counted in identical geometry on a support above the crystal of a scintillation well counter.

Spot Size: To determine the effect of wet spot size on chromatogram streaking, five 1 × 10 cm strips were spotted with either 1, 2, 3, or 5 μl of Tc-99m DTPA and developed immediately in acetone. Strips were then dried, cut into 1-cm segments, and counted as before.

Drying Technique: To determine the influence of strip drying technique on analytical results, 1 × 5 cm strips were spotted with 3 μl of various technetium-99m complexes (MDP, DTPA, PPi, or GH) and dried according to one of the following methods before development in acetone: (1) nitrogen-purged glove box for 20 min, (2) room air for 20 min, (3) hot air blower for 20 sec, or (4) hot air blower for 20 sec plus room air for 20 min. Five chromatograms were run for each condition. Strips were dried and counted to determine the activity in each half of the strip.

Spot Placement: Several chromatographic strips (1 × 5 cm) were prepared with origin marks at 2 mm, 4 mm, and 8 mm from the bottom of each strip. Four μl of pertechnetate was spotted at each origin mark and immediately developed in saline. Saline vials were prepared by pipetting exactly 0.3 ml of saline into each test tube producing a fluid level 3 mm deep. Five samples were run for each condition. Chromatograms were dried, cut, and counted with a scintillation counter to determine the activity in each half of the strip.

Results and Discussion

Table 1 indicates the effect on analytical results for chromatograms of several technetium complexes developed in acetone with wet or dry spots. It is evident that the results obtained with each method are significantly different. Wet spots produce substantial amounts of activity in the solvent front half of the strip. Since the ITLC-SG/acetone system is designed to measure free pertechnetate impurity, wet spot chromatograms yield results that indicate grossly impure radiopharmaceuticals. This, in fact, is an artifact. When spots are thoroughly dried before development the results indicate less than 1% pertechnetate impurity. The explanation for these differences is quite simple. Technetium complexes are very soluble in aqueous solvent like saline and poorly soluble in organic solvent like acetone. If spots are not dried completely, the acetone mixes with the water in the spot during development and causes the spot to streak up the chromatogram. The degree of streaking depends to some extent upon the solubility of the technetium complex in the water/acetone mixture and on its degree of adsorption by the silica gel.

TABLE 1. Effect of Wet Spot Versus Dry Spot on Chromatography Results of Tc-compounds in ITLC-SG/Acetone System

Agent	Percent Activity*			
	Wet spot		Dry spot	
	Origin	Solvent front	Origin	Solvent front
Tc-GH	74.01	25.99	99.81	0.19
Tc-DTPA	60.04	39.96	99.70	0.30
Tc-PPi	85.37	14.63	99.39	0.61
Tc-MDP	82.60	17.40	99.82	0.18

*Mean of five determinations

This explains the differences in activity distribution observed between the different complexes run with wet spots.

Figure 2 demonstrates further that the amount of streaking for a particular complex increases with spot size and is minimized to insignificant levels when the spot is completely dry. In this experiment a strip of 10-cm length is used to demonstrate that the amount of "artifactual pertechnetate" measured is minimized or eliminated if a longer strip is used or if a smaller spot is applied. Thus, if a 10-cm strip is used for the chromatographic analysis, only the 5-μl wet spot chromatogram yields significant amounts of activity in the top portion of the strip. However, if 5-cm strips are used, as is commonplace with miniaturized chromatography systems, and cut at the 2.5-cm mark, every wet spot sample yields substantial activity in the top portion of the strip. Only the dried spot strip produces valid results.

Table 2 indicates the effect that spot drying conditions have on chromatography results in acetone for each Tc-99m complex. All samples dried under a nitrogen atmosphere yielded results of 1% or less of pertechnetate impurity. Samples dried in room air for the same time interval yielded results similar to nitrogen drying for DTPA, PPi, and GH complexes but showed evidence of significant degradation with the MDP samples. This increase in solvent front activity due to pertechnetate impurity is caused by air oxidation of the MDP samples during the drying process. MDP-A showed substantially greater degradation than MDP-B. This is attributed to the much smaller amount of tin (II) in MDP-A (0.19 mg) compared to MDP-B (0.45 mg) thus providing less protection from air oxidation. When both MDP samples are dried immediately after spotting with a hot air blower, the chromatography results are identical to those obtained under nitrogen. Similar results are also obtained if these samples are immediately dried but exposed to room air for 20 min before development. These results show that rapid removal of water from the spot is the most important step in preventing sample oxidation and that once a sample is dry, very little if any additional oxidation takes place.

Thus, rapid air drying of the spot is just as effective

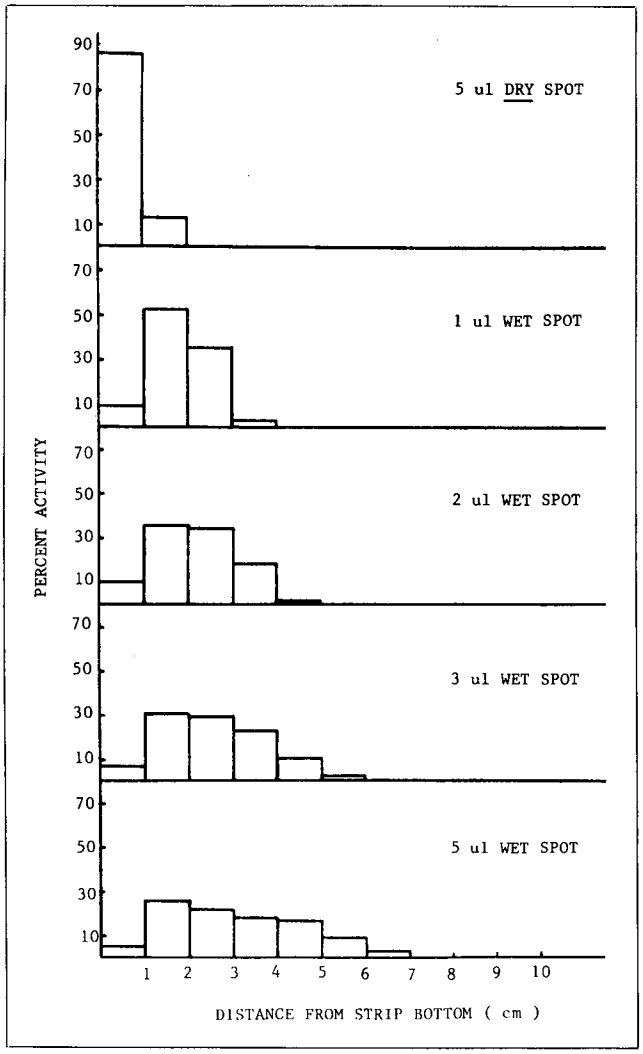

FIG. 2. Chromatogram streaking of Tc-99m DTPA in the ITLC-SG/acetone system as a function of wet spot size.

as drying in a nitrogen atmosphere, since no significant artifacts are produced by this method. No substantial degradation of DTPA, PPi, or GH is seen when these samples are dried in room air. We attribute this to either greater stability of the Tc-complex in the case of DTPA or GH, or to large amounts of stannous ion (2.1 mg per vial) in the case of PPi.

Table 3 demonstrates the effect produced during chromatography when the sample is spotted in different positions at the strip origin. It is important for all chromatography procedures that the entire spot be above the level of the solvent, allowing the solvent to migrate through the spot. If the spot is positioned so that it soaks in the solvent, poor migration of soluble species occurs up the strip. Since the saline system is used to measure hydrolyzed-reduced technetium impurity, a strip whose spot enters the saline will produce artifactual impurity. In this experiment, pertechnetate was used since it is freely soluble in saline and will migrate completely to the solvent front. Other soluble species, such as Tc-complexes, will behave in a similar manner. A 4-μl sample was used, which produces a 4-mm diameter spot on the strip. The solvent level is 3 mm deep so that a spot at 2 mm from the strip bottom is about 75% submerged, creating a large artifact in the chromatogram, with additional activity leaching into the solvent as well. A spot at 4 mm from the bottom is about 25% submerged and yields a smaller but significant artifact. The spot at 8 mm is well above the saline level and produces good chromatographic results.

On several occasions, technologists at our institution have reported high percentages of hydrolyzed-reduced technetium activity during daily chromatography procedures. In all instances we were able to attribute this to technical artifacts from too large a spot or one placed too close to the strip bottom. Reports from other hos-

TABLE 2. Effect of Spot Drying Conditions on Chromatographic Analysis of Tc-99m Compounds with ITLC-SG/Acetone System

Drying conditions	Percent Activity*									
	MDP-A		MDP-B		PPi		DTPA		GH	
	O	SF	O	SF	O	SF	O	SF	O	SF
Room air 20 min	81.4	18.6	96.0	4.0	99.5	0.5	99.8	0.2	99.1	0.9
Nitrogen 20 min	100.0	0	100.0	0	99.4	0.6	99.9	0.1	98.9	1.1
Hot air 20 sec	99.9	0.1	99.8	0.2	99.4	0.6	99.7	0.3	99.8	0.2
Hot air 20 sec plus Room air 20 min	99.6	0.4	99.6	0.4	99.5	0.5	99.8	0.2	99.3	0.7

*Mean of 5 determinations
O = origin.
SF = solvent front.

TABLE 3. Effect of Spot Placement at Origin on Chromatography Results Using ITCL-SG/Saline System

Distance from Strip bottom	Percent Activity*		
	Origin	Solvent front	Solvent
2 mm	37.43	47.39	15.18
4 mm	6.89	92.44	0.67
8 mm	0.10	99.90	0

*Mean of five determinations; 4-μl spot of sodium pertechnetate

pitals have noted high pertechnetate impurity, which was found to be caused by allowing strips to dry in room air for several minutes. This method may yield adequate results for technetium radiopharmaceuticals that contain large amounts of tin (II) but is not recommended since air oxidation will always occur to some extent, thus producing a false measure of the pertechnetate impurity actually present in the vial.

Conclusions

Miniaturized chromatography systems provide a rapid, easy method to assess the radiochemical purity of technetium radiopharmaceuticals, provided that strict techniques are followed. Particular attention should be directed towards the following for accurate and precise results:

- ☐ prepare strips with origins 1 cm from the bottom;
- ☐ apply small spots, preferably 1 to 3 μl in size, which remain above the solvent level; and
- ☐ for strips run in acetone, dry the spots immediately and completely before development using either nitrogen or a hot air stream.

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