Technical Parameters Associated with Miniaturized Chromatography Systems

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The technical parameters associated with miniaturized chromatography systems for several Tc-99m preparations were evaluated. These parameters included effects of radiopharmaceutical spot size; effects of drying time between spotting and strip development; technologist's degree of chromatographic experience; and effect of incorrect strip counting on the radiochemical evaluation of Tc-99m radiopharmaceuticals. Results indicate that small radiopharmaceutical samples (2 μl) should be spotted on the chromatography strips and these strips should then be developed immediately. A technologist's degree of chromatographic experience has little effect on the radiochemical evaluation of the radiopharmaceuticals as long as the proper procedure is used. Incorrect counting of the sectioned chromatography strips produces large errors in the radiochemical evaluation of a specific Tc-99m radiopharmaceutical.

Quality control procedures for Tc-99m radiopharmaceuticals including chromatographic radiochemical evaluations are becoming an increasingly important aspect of nuclear medicine. With the advent of miniaturized chromatography procedures (*1-3*) including commercial kits, these procedures are becoming very quick and also relatively easy to perform. However, variations in the results obtained when using miniaturized chromatography systems have been observed by some investigators. In order to more fully understand these variations, we investigated multiple technical parameters associated with the chromatographic quality control procedures used in our laboratory (*2*). Parameters included the effect of various radiopharmaceutical spot sizes and drying times before strip development on the radiochemical evaluation of [^{99m}Tc] pertechnetate and hydrolized reduced Tc-99m (Tc-HR). In addition, the effect of a technologist's chromatographic experience and the effect of improperly counting chromatography strips on the radiochemical evaluation of a radiopharmaceutical preparation were evaluated.

Materials and Methods

The miniaturized chromatographic procedure utilized was developed in our laboratory (*2*). Miniaturized Whatman 31 ET paper chromatography strips (Whatman Chromatography Products, Clifton, NJ) (1 cm × 6 cm) were used with acetone as the solvent to quantify free [^{99m}Tc] pertechnetate in Tc-99m radiopharmaceuticals. With this system, free pertechnetate migrated in close proximity to the solvent front, whereas the labeled radiopharmaceutical and Tc-HR remained at the origin. By cutting the strip at the center (center pencil line) and counting each section (marked 1 and 2), the level of free pertechnetate was determined. The level of Tc-HR or reduced Tc-99m not bound to the radiopharmaceutical was determined by using Gelman silica gel–instant thin layer chromatography (Gelman Instrument Co., Ann Arbor, MI) (1 cm × 6 cm) and 0.9% sodium chloride. With this system, the Tc-HR remained at the origin whereas the remaining activity migrated with the solvent front. Again, by sectioning the strip at the center pencil line and counting each section (marked 3 and 4), the level of Tc-HR was easily determined. The general procedure is fully explained in Table 1. (Reference 2 notes a more complete explanation of the chromatography system.)

The effect of spot size and drying time before chromatographic development on the radiochemical evaluation of free [^{99m}Tc] pertechnetate and of Tc-HR was determined in the following manner. For the evaluation of free [^{99m}Tc] pertechnetate, excess pertechnetate was added to Tc-99m-Sn-diphosphonate and Tc-99m-Sn DTPA preparations (Diagnostic Isotopes, Bloomfield, NJ) so that the resultant preparations contained between 20–35% free pertechnetate in a total volume of 2.0 ml. Different spot sizes of each preparation, ranging from 2 to 10 μl, were spotted on five miniaturized 31 ET strips and immediately developed in acetone. In order to evaluate the radiopharmaceutical spot drying effect, the radio-

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TABLE 1. Determining Free [^{99m}Tc] Pertechnetate and Tc-HR in Tc-99m Radiopharmaceuticals

1. Add approximately 1 ml of acetone to a 10-ml glass vial and 1 ml of 0.9% NaCl to a 10-ml glass vial.
2. Spot radiopharmaceutical on bottom pencil line of 31 ET paper chromatography and ITLC-SG chromatography strips.
3. Develop 31 ET paper strip in acetone solvent and ITLC-SG strip in 0.9% NaCl solvent until solvent front migrates to top pencil line.
4. Cut strips at center pencil line for both chromatography strips into sections 1, 2, 3, and 4.
5. Count all sections for activity using a gamma counter and subtract background:

$$\%\ \text{free}\ [^{99m}\text{Tc}]\ \text{pertechnetate} = \frac{(\text{net counts section 2})}{(\text{net counts section 2}) + (\text{net counts section 1})} \times 100$$

$$\%\ \text{Tc-HR} = \frac{(\text{net counts section 3})}{(\text{net counts section 3}) + (\text{net counts section 4})} \times 100$$

pharmaceutical preparations were spotted on 31 ET strips (2 μl) and developed in acetone at various time intervals after spotting (from 0 to 10 min). Again, for each specific time interval five individual determinations were performed.

For the evaluation of Tc-HR, stannous chloride was added to a [^{99m}Tc] pertechnetate solution so that the resultant mixture would contain between 15–25% Tc-HR. For each spot size (2–10 μl) and for each drying time interval after spotting (0–5 min), the radiopharmaceutical mixture was added to five ITLC-SG strips and developed in 0.9% sodium chloride.

The effect of a technologist's amount of chromatography experience on the radiochemical evalution of a Tc-99m-Sn-DTPA preparation was also determined. Excess [^{99m}Tc] pertechnetate and stannous chloride were added to a Tc-99m-Sn-DTPA preparation so that each radiochemical component—free pertechnetate, Tc-HR, and Tc-99m-Sn-DTPA—would contain between 30–40% of the total activity. Each of the three operators then spotted the radiopharmaceutical mixture (2 μl spots) on five ITLC-SG strips; these strips were immediately developed in acetone and sodium chloride, respectively.

The effect of deadtime when counting the strip on the evaluation of free pertechnetate was determined by counting a sectioned chromatography strip, containing approximately 14% free pertechnetate, at various distances from a scintillation well counter. The distances ranged from 1.5 to 19 cm from the well detector. Five repeated counts were performed at each specified distance.

All data were statistically analyzed by determining means and standard deviations. A Student's t-test (*4*) was utilized to find statistically significant differences between experimental mean values.

Results

The results of determining the effect of spot size and spot drying on the radiochemical evaluation of free pertechnetate in radiopharmaceutical preparations are shown in Tables 2 and 3. As indicated in Table 2, a decrease in the free pertechnetate level was observed when the size increased from 2 to 5 μl for Tc-99m-Sn-DTPA ($p < 0.10$) and for Tc-99m-Sn-diphosphonate ($p < 0.05$). No significant differences were observed between the 5 μl and 10 μl spot sizes. In addition, with increasing spot sizes (from 2–5 μl), the variation as expressed by the standard deviation increased.

The effect of spot drying prior to development on the level of free pertechnetate in a radiopharmaceutical preparation is shown in Table 3. For the Tc-99m-Sn-DTPA preparation, a gradual significant decrease in free pertechnetate was noted with increased drying time. Statistical analysis of the data indicated that a drying time of 10 min results in significantly less pertechnetate than all other drying time intervals ($p < 0.001$). In addition, a 3- or 5-min drying time interval also results in significantly less pertechnetate than shorter drying time intervals ($p < 0.01$). With Tc-99m-Sn-diphosphonate there is an initial significant increase of free pertechnetate from immediate strip development to a 3-min drying time interval ($p < 0.05$), followed by a statistically significant decrease in the pertechnetate levels at a drying time interval of 5 min ($p < 0.05$) and 10 min ($p < 0.001$).

The effects of spot size and drying time after spotting on the radiochemical determination of Tc-HR are shown in Tables 4 and 5, respectively. As indicated in Table 4, increasing spot size did not significantly change the mean Tc-HR levels. However, the degree of variation as expressed by the standard deviation generally increased

TABLE 2. Effect of Varying Spot Sizes on Chromatographic Determination of Free Pertechnetate in Tc-99m-DTPA and Tc-99m-Sn-Diphosphonate Using 31-ET Paper and Acetone

Spot size (μl)	Free [^{99m}Tc] pertechnetate (mean percent ± s.d.) Tc-99m-Sn-DTPA	Tc-99m-Sn-diphosphonate
2	24.9 ± 0.6	32.8 ± 1.0
5	21.3 ± 3.7	28.3 ± 3.7
10	25.3 ± 3.5	28.8 ± 2.7

TABLE 3. Effect of Time between Strip Spotting and Developing on Chromatographic Determination of Free Pertechnetate Using 31 ET Paper and Acetone

Time between strip spotting and developing	Free [^{99m}Tc] Pertechnetate (mean percent ± s.d.) Tc-99m-Sn-DTPA	Tc-99m-Sn-diphosphonate
Immediately after spotting	19.0 ± 1.5	24.8 ± 1.4
1 min	18.5 ± 0.7	25.8 ± 1.3
3 min	16.9 ± 0.3	27.5 ± 0.6
5 min	15.2 ± 0.6	26.3 ± 0.6
10 min	6.0 ± 0.7	16.5 ± 1.6

TABLE 4. Effect of Varying Spot Sizes on Chromatographic Determination of Tc-HR Using ITLC-SG and 0.9% Sodium Chloride

Spot Size (μl)	Time between Spotting and Strip Development	Percent Tc-HR (Mean percent ± s.d.)
2.0	Immediate	30.1 ± 0.2
5.0	Immediate	29.4 ± 1.7
10.0	Immediate	29.1 ± 1.6

TABLE 5. Effect of Time between Strip Spotting and Strip Developing on Chromatographic Determination of Tc-HR Using ITLC-SG and 0.9% Sodium Chloride

Time between strip spotting and developing	Tc-HR (mean percent ± s.d.)
Immediately after spotting	23.3 ± 0.4
1 min	23.1 ± 0.8
3 min	17.4 ± 1.2
5 min	15.1 ± 0.5

with increasing spot size. The effect of spot drying on the Tc-HR levels is shown in Table 5. Significant decreases in the levels of Tc-HR ($p < 0.05$) were observed when the spot was dried for more than 1 min. Variations also rose with increasing drying times.

The effect an operator's experience had on the radiochemical evaluation of a Tc-99m radiopharmaceutical preparation is shown in Table 6. No significant differences were observed between the three operators in the chromatographic evaluation of Tc-99m-Sn-DTPA, [^{99m}Tc] pertechnetate, and Tc-HR with one exception. A statistically significant difference ($p < 0.05$) was observed between the experienced operator and the other two operators for Tc-HR; however, the mean difference observed was only 1.3%.

Table 7 shows the erroneous results that can be obtained if the chromatography strips are counted too close to the well detector. A completely false overestimation of the free pertechnetate level in a radiopharmaceutical preparation was obtained—if the strips were counted in close proximity to the well (13.9 and 38.2% free pertechnetate at 19 cm and 1.5 cm from the well, respectively).

Discussion

We believe that the most prominent error produced when using the miniaturized chromatography system involves counting the individual strip sections too closely to the scintillation well detector. Very large overestimations of free pertechnetate and Tc-HR can occur as a result of instrument deadtime (Table 7). As a general rule in our laboratory, we collect between 30,000–50,000 counts /30 sec for the high activity strip section. We do this by moving the strip either closer to or farther from the well, depending upon the activity (counts/30 sec) recorded.

Radiopharmaceutical spot size is an important consideration when performing chromatography, particularly when 31 ET paper is used (Table 2). Because variation as expressed by the standard deviation is minimal with a 2 μl spot size, we recommend this spot size for the miniaturized chromatography system.

Variations in the free pertechnetate levels were obtained when assessing various drying time intervals in Tc-99m-Sn-DTPA and Tc-99m-Sn-diphosphonate preparations (Table 3). The pertechnetate levels initially rose with increased drying time and then decreased for Tc-99m-Sn-diphosphonate, whereas the pertechnetate levels continuously decreased with increased drying time for the Tc-99m-Sn-DTPA preparation. One must realize that the Tc-99m-Sn-diphosphonate preparation is a much weaker complex than Tc-99m-Sn-DTPA; therefore, oxidation by air with the subsequent production of free pertechnetate can occur much more readily with the diphosphonate. Binding between the pertechnetate and the paper chromatography also occurs as evidenced by the reduction in the free pertechnetate level with increased drying time. Increased drying time also produces a decrease in the levels of Tc-HR (Table 5). Again, oxidation by air is probably responsible for this. Because of the oxidation problem, as well as the problem of radiopharmaceutical binding to the chromatography strips, we recommend that miniaturized chromatography strips be developed immediately after radiopharmaceutical spotting.

The operator's degree of chromatography experience

TABLE 6. Chromatographic Evaluation of a Tc-99m-Sn-DTPA Mixture Using Three Nuclear Medicine Technologists

Technologists	Chromatographic Evaluation (mean percent ± s.d.) Tc-99m-Sn-DTPA	[^{99m}Tc] pertechnetate	Tc-HR
Most experienced	29.0 ± 2.5	35.4 ± 4.3	35.6 ± 0.5
Experienced	29.1 ± 2.0	34.0 ± 2.1	36.9 ± 0.7
Least experienced	32.0 ± 2.8	32.4 ± 2.9	35.6 ± 0.4

TABLE 7. Effect of Chromatography Strip Placement when Counting on the Evaluation of Free Pertechnetate in a Radiopharmaceutical Preparation (the free [^{99m}Tc] pertechnetate level in the preparation was approximately 14%)

Distance from top of well to strip (in cm)	Total count (counts/30 sec)	Free [^{99m}Tc] pertechnetate (mean percent ± s.d.)
19.0	30,000	13.9 ± 0.1
15.0	50,000	14.1 ± 0.3
10.5	100,000	15.4 ± 0.4
6.0	300,000	16.4 ± 0.9
1.5	800,000	38.2 ± 0.8

did not really matter in the radiochemical evaluation of the specified radiopharmaceutical (Table 6). It should be noted, however, that even the most experienced person followed a detailed, written protocol. Our results point to the fact that a precise chromatographic procedure must be established and followed if miniaturized chromatography strips are used. In our laboratory, we utilize small radiopharmaceutical sample spots (approximately 2 μl) and develop the strip immediately after spotting. With this technique, we have been able to obtain excellent clinical correlations between radiochemical purity of bone-seeking radiopharmaceuticals and image quality (*5,6*). We currently use this technique in our daily Tc-99m radiopharmaceutical quality control program and find the procedure to be rapid, easy, reproducible, and inexpensive.

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