Rapid Quality Control of Technetium-99m-Tetrofosmin: Comparison of Miniaturized and Standard Chromatography Systems

Monica C. Geyer, A. Michael Zimmer, William G. Spies, Stewart M. Spies and Robert C. Hendel

Section of Nuclear Cardiology, Department of Nuclear Medicine, Northwestern University Medical Center, Chicago, Illinois

Objective: In this paper, we evaluate the use of miniaturized ITLC-SG strips (10 cm × 1 cm) with the same solvent system of methylene chloride:acetone (65:35) to reduce the time required to perform the quality control procedure for ^{99m}Tc-tetrofosmin. The potential problems inherent in the standard chromatography system were investigated as to the magnitude of their effects on the miniaturized system.

Methods: The migration of the radiochemical components of ^{99m}Tc-tetrofosmin was evaluated using multiple miniature and standard strip sizes. Following optimization of the miniaturized system, radiochemical purity evaluations were performed simultaneously on 112 ^{99m}Tc-tetrofosmin preparations using both chromatography systems and the radiochemical purity results compared.

Results: Initially, similar migration of radiochemical components was found using both chromatography systems. Results of the radiochemical purity evaluations of ^{99m}Tc-tetrofosmin preparations were similar with both chromatography methods, with a mean difference of 1.3% ± 1.5%. Differences in radiochemical purity results between the two chromatography systems were less than 2% in the majority of samples. In no instance was a preparation acceptable with one chromatography system and not acceptable with the other.

Conclusion: The results indicate that the miniaturized chromatography system is as effective in evaluating the radiochemical purity of ^{99m}Tc-tetrofosmin as the standard quality control procedure, with a substantial reduction in the time required to perform the procedure.

Key Words: technetium-99m-tetrofosmin; radiochemical purity testing; miniaturized chromatography system

J Nucl Med Technol 1995; 23:186–189

Recently a new myocardial perfusion imaging agent, ^{99m}Tc-tetrofosmin (Myoview®, Amersham Healthcare, Arlington Heights, IL) was developed. This new agent demonstrates excellent imaging properties and a similar diagnostic accuracy to ^{201}Tl-thallous chloride (*1*). Following preparation of this new agent, the manufacturer's recommended quality control procedure was utilized to determine the radiochemical purity using a single-strip chromatography system (*2*). The manufacturer-recommended chromatography system requires almost 30 min for completion. The current study was undertaken to determine whether a miniaturized chromatography strip might yield more rapid assessments of radiochemical purity of ^{99m}Tc-tetrofosmin preparations without altering the accuracy of the procedure.

For correspondence and reprints contact: Monica C. Geyer, CNMT, Section of Nuclear Cardiology, Northwestern Memorial Hospital, 250 E. Superior St., Chicago, IL 60611.

MATERIALS AND METHODS

The manufacturer's recommended chromatography system uses ITLC-SG paper (Gelman Instruments, Ann Arbor, MI) that are cut into 2 × 20 cm with the origin and solvent front drawn 3 cm and 2 cm from the bottom and top of the strip, respectively. Two cut lines are drawn 3 cm and 12 cm above the origin. The strip is developed in a solvent system of methylene chloride:acetone (65:35). Following strip development free pertechnetate migrates to the solvent front, hydrolyzed reduced ^{99m}Tc remains at the origin, and ^{99m}Tc-tetrofosmin migrates to the middle section of the strip (*2*). The miniaturized chromatography procedure formulated by our lab is shown in Table 1. The parameters for the miniaturized system remain the same as with the standard system with the exception of strip size and radiopharmaceutical spot size.

An illustration of standard and miniaturized chromatography strips, with appropriate cut lines, is shown in Figure 1. The migration of the various radiochemical components of ^{99m}Tc-tetrofosmin was evaluated simultaneously using both standard and miniaturized strips (four strips each). Chromatography developing tanks were initially saturated with the required solvent system. The strips were spotted (10 μl for standard and 5 μl for miniaturized) and immediately placed

TABLE 1
Miniaturized Chromatography Procedure for ^{99m}Tc-Tetrofosmin

1. Saturate developing chamber with solvent mixture (methylene chloride:acetone (65:35).
2. Spot 5 μl of radiopharmaceutical at origin of miniaturized chromatography strip.
3. Immediately develop chromatography strip in solvent mixture. Do not allow spot to dry.
4. Allow solvent to migrate to solvent front line (approximately 4 min).
5. Remove strip and cut, at both cut lines, into 3 sections.
6. Count each section for activity using appropriate counting instrumentation.
7. Calculate labeling efficiency as follows:

$$\% \text{ Labeling} = \left[\frac{\text{Net counts of middle section}}{\text{Net counts of all sections}}\right] \times 100$$

in developing tanks. When the solvent migrated to the solvent front line, the strips were removed from the tanks and cut into 15 equal sections, 1.0 cm per section for standard and 0.5 cm per section for miniaturized strips. The sections were counted for activity on a well detector system interfaced to a multichannel analyzer (D. S. Davison, New Haven, CT) and activity distribution curves were generated.

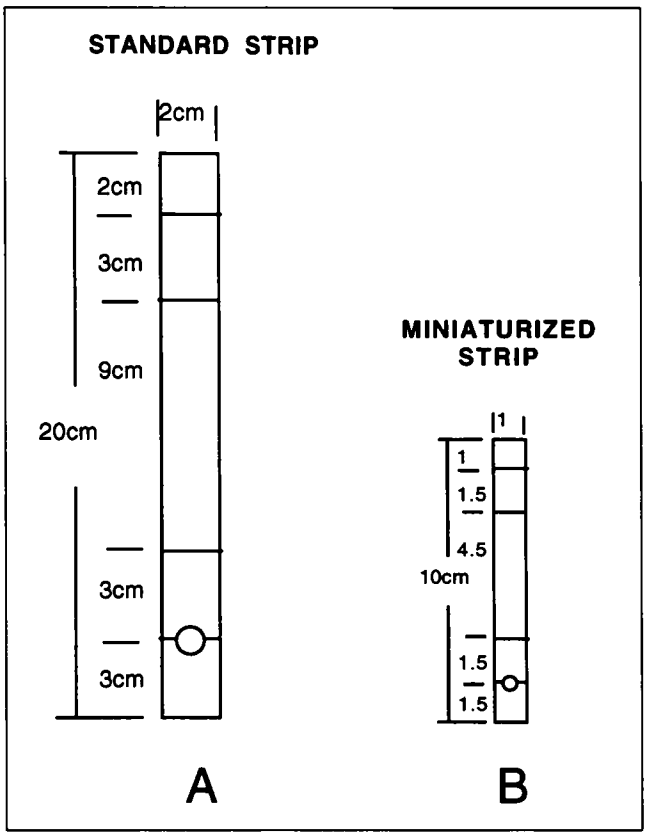

FIGURE 1. Dimensions of (A) standard ITLC-SG chromatography strip and (B) miniaturized ITLC-SG strip.

The development time required for both the standard and miniaturized strips was noted. The migration of free pertechnetate was evaluated on both strip sizes by spotting [^{99m}Tc]-pertechnetate, developing the strips as described above and generating activity distribution curves.

Some potential problems related to the chromatography system include the effect of altering solvent ratios (methylene chloride:acetone) and radiopharmaceutical spot size on the migration of ^{99m}Tc-tetrofosmin. These potential problems were also investigated on miniaturized strips.

The effect of altering solvent ratios (methylene chloride: acetone), including solvent ratios of 70:30, 65:35 and 60:40, on the migration of ^{99m}Tc-tetrofosmin was assessed by spotting duplicate miniaturized strips and immediately placing them in developing tanks that had been saturated with the respective solvent ratio. When solvent migration was complete, the strips were removed from the tanks and cut into 15 equal sections. The sections were counted for activity on a well detector system and activity distribution curves generated for each ratio.

The effect of increasing radiopharmaceutical spot size was evaluated by spotting duplicate miniaturized strips with 2.5, 5.0 and 10.0 μl ^{99m}Tc-tetrofosmin. The strips were then placed into developing tanks, allowed to develop to the solvent front line, removed from the solvent, cut into 15 equal sections and counted for activity. Activity distribution curves were generated for each specific radiopharmaceutical spot size.

The miniaturized chromatography system was compared to the standard chromatography system by evaluating the radiochemical purity of 112 ^{99m}Tc-tetrofosmin preparations. The paired strips were spotted and immediately placed in developing tanks that had been saturated with the required solvent system. Following solvent migration, the strips were removed and cut at the indicated cut lines. The three sections were then counted for activity on a well detector system. From the activity in each section, the radiochemical purity of each specific preparation was calculated. The differences in radiochemical purities of ^{99m}Tc-tetrofosmin using the standard and miniaturized chromatography systems was calculated for each specific preparation. The data were summarized by calculating mean differences in radiochemical purity and standard deviations between each specific chromatography system.

RESULTS

The initial evaluation of the migration (Rf value) of the various radiochemical species, using both the standard and miniaturized systems, are shown in Figures 2 and 3, respectively. Free [^{99m}Tc]-pertechnetate migrated to the solvent front and the hydrolyzed reduced ^{99m}Tc component remained at the origin with both sized strips. Technetium-99m-tetrofosmin showed good separation from both free and hydrolyzed reduced ^{99m}Tc in both systems, with an Rf value of approximately 0.5.

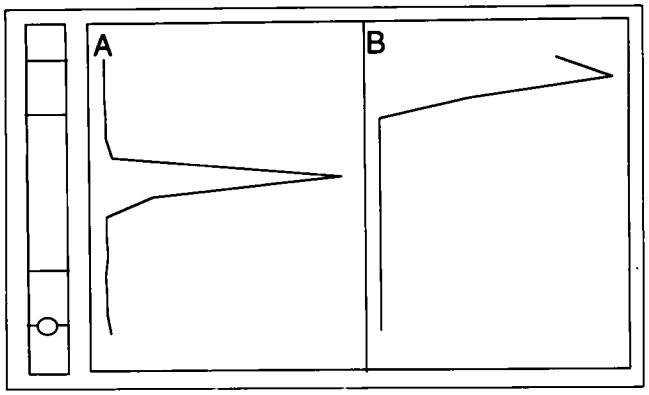

FIGURE 2. Chromatographic evaluation of (A) ^{99m}Tc-tetrofosmin and (B) [^{99m}Tc]-pertechnetate using standard chromatography system.

We evaluated the time required to develop each type of chromatography strip. The average time required to develop the standard strip was 28 min while the time needed for the miniaturized strip was approximately 4 min, a more than six-fold reduction in developing time due to strip miniaturization.

The effect of slightly altering the solvent ratios on the migration of ^{99m}Tc-tetrofosmin using miniaturized strips is shown in Figure 4. A lower acetone concentration (70:30 v/v methylene chloride:acetone), resulted in a decrease in the migration of ^{99m}Tc-tetrofosmin (Rf = 0.4). Conversely, a higher acetone concentration (60:40 v/v methylene chloride: acetone), resulted in an increase in the migration of ^{99m}Tc-tetrofosmin (Rf = 0.75). With standard solvent concentrations (65:35 v/v methylene chloride:acetone), ^{99m}Tc-tetrofosmin migrated with an Rf value of 0.5. The changes of the migration of ^{99m}Tc-tetrofosmin caused by the slight alterations in the solvent ratios could possibly result in inaccurate radiochemical purity evaluations, with an overestimation of hydrolyzed reduced ^{99m}Tc using a 70:30 methylene chloride:acetone solvent ratio or an overestimation of free pertechnetate using a 60:40 methylene chloride:acetone solvent ratio.

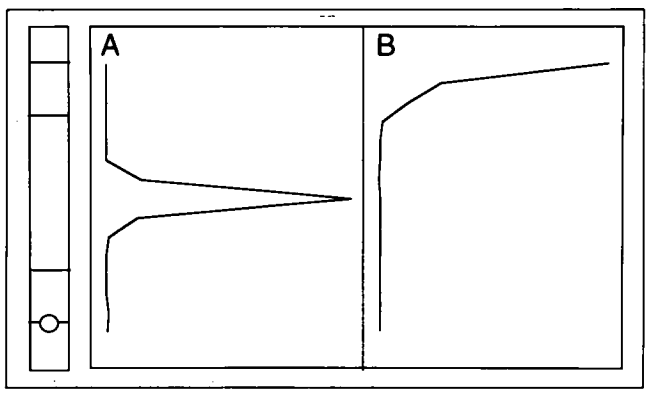

FIGURE 3. Chromatographic evaluation of (A) ^{99m}Tc-tetrofosmin and (B) [^{99m}Tc]-pertechnetate using miniaturized chromatography system.

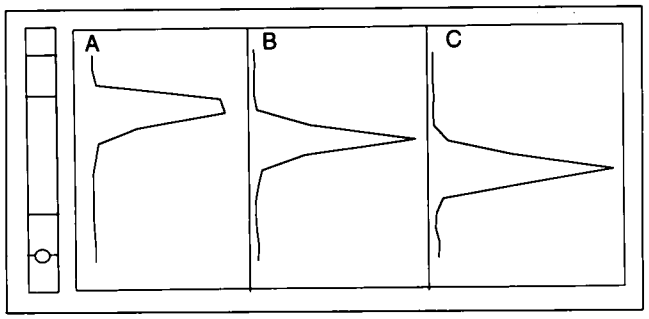

FIGURE 4. Effect of altering methylene chloride:acetone solvent ratio on the migration of ^{99m}Tc-tetrofosmin: (A) methylene chloride: acetone (60:40 ratio); (B) methylene chloride:acetone (65:35 standard ratio); and (C) methylene chloride:acetone (70:30 ratio).

The effects of altering radiopharmaceutical spot size on the migration of ^{99m}Tc-tetrofosmin are shown in Figure 5. With the standard spot size (5 μl), the most acceptable separation of ^{99m}Tc-tetrofosmin from other radiochemical species was achieved. However, altering the radiopharmaceutical spot size, from 2.5 μl to 10 μl, affected the migration of ^{99m}Tc-tetrofosmin. The 2.5 μl radiopharmaceutical spot size decreased the migration of ^{99m}Tc-tetrofosmin and could overestimate ^{99m}Tc hydrolyzed reduced levels. The 10.0 μl spot size increased the migration of ^{99m}Tc-tetrofosmin, thus overestimating free pertechnetate levels in these preparations. Therefore, alterations of spot size may lead to inaccurate assessments in radiochemical purity of ^{99m}Tc-tetrofosmin.

Radiochemical purity results of 112 ^{99m}Tc-tetrofosmin preparations using both chromatography techniques were similar (Fig. 6) with a mean difference in radiochemical purity of 1.3% ± 1.5% (s.d.). Differences of radiochemical purity were less than 2% in 92 of 112 paired samples. When differences in radiochemical purity were greater than 2%, a random distribution was noted indicating that the variability of results was not due to systematic errors. In all instances, determinations of acceptable radiochemical purity (>90%) were concordant between the miniaturized and standard chromatography systems.

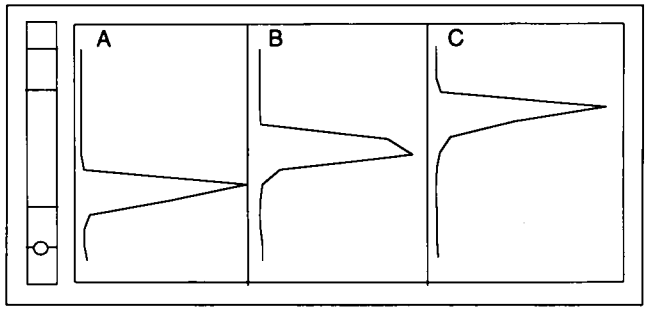

FIGURE 5. Effect of altering radiopharmaceutical spot size on the migration of ^{99m}Tc-tetrofosmin: (A) 2.5 μl spot; (B) 5.0 μl spot; and (C) 10.0 μl spot.

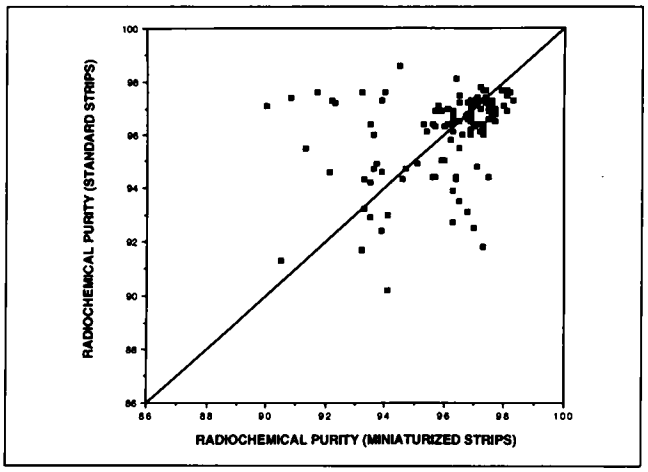

FIGURE 6. Comparison of radiochemical purity of ^{99m}Tc-tetrofosmin using standard ITLC-SG chromatography system versus miniaturized ITLC-SG chromatography system.

DISCUSSION

Miniaturized chromatography procedures have been utilized to evaluate radiochemical purity of various ^{99m}Tc (*3,4*) and iodinated radiopharmaceuticals (*5*), as well as newer radiopharmaceuticals (*6*). In addition, technical parameters and common errors associated with miniaturized chromatography systems previously have been reported (*7–9*).

The current study reveals that miniaturization of the chromatography system significantly reduces the time required for the quality control of ^{99m}Tc-tetrofosmin (28 min for standard versus 4 min for miniaturized) without compromising accuracy. The conventional quality control method used to assess radiochemical purity of ^{99m}Tc-tetrofosmin utilizes ITLC-SG paper (20 cm × 2 cm) in a solvent system of methylene chloride:acetone (65:35) (*2*). Although the procedure is well defined, it is time-consuming (exceeding 25 min), causing a potential deterrent to the clinical use of ^{99m}Tc-tetrofosmin. Our laboratory evaluated miniaturized chromatography strips (1 cm × 10 cm) in order to reduce the time required to perform the quality control procedure. Utilizing miniaturized chromatography strips has reduced the time required to perform the quality control procedure to less than 5 min.

When utilizing the miniaturized chromatography system, it should be stressed that the composition of the solvent mixture of methylene chloride:acetone (65:35) is important. Too much acetone will increase migration of ^{99m}Tc-tetrofosmin, while too little has an opposite effect. The radiopharmaceutical spot size, 5 μl, is also important in the accurate assessment of the radiochemical purity of ^{99m}Tc-tetrofosmin preparations. Smaller or larger sample volumes may significantly alter the migration of ^{99m}Tc-tetrofosmin, resulting in a false estimation of radiochemical purity.

In conclusion, the results of the current study demonstrate that a miniaturized chromatography system is effective in evaluating the radiochemical purity of ^{99m}Tc-tetrofosmin. Furthermore, these radiochemical purity results were available within 5 min, resulting in a six-fold time savings over the standard chromatography technique.

ACKNOWLEDGMENT

This research was supported in part by a grant from Amersham Healthcare, Arlington Heights, IL.

REFERENCES

1. Zaret BL, Rigo P, Wackers FJT, et al. Myocardial perfusion imaging with technetium-99m tetrofosmin: comparison to thallium-201 imaging and coronary angiography in a phase III multicenter trial. *Circulation*; in press.
2. Tetrofosmin package insert. Arlington Heights, IL: Amersham Healthcare; October 1991.
3. Zimmer AM, Majewski W, Spies SM. Rapid miniaturized chromatography for Tc-99m IDA agents: comparison with gel chromatography. *Eur J Nucl Med* 1982;7:88–91.
4. Zimmer AM, Pavel DG. Rapid miniaturized chromatographic quality-control procedures for Tc-99m radiopharmaceuticals. *J Nucl Med* 1977; 18:1230–1233.
5. Zimmer AM, Pavel DG. Rapid miniaturized chromatographic quality control procedures for iodinated radiopharmaceuticals. *Amer J Hosp Pharm* 35:426–428, 1978.
6. Zimmer AM, Spies SM. Quality control procedures for newer radiopharmaceuticals. *J Nucl Med Technol* 1991;19:210–215.
7. Webber D, Zimmer AM, Spies SM. Common errors associated with miniaturized chromatography. *J Nucl Med Technol* 1989;11:66–68.
8. Kowalsky RJ, Creekmore JR. Technical artifacts in chromatographic analysis of Tc-99m radiopharmaceuticals. *J Nucl Med Technol* 1982;10: 15–19.
9. Taukulis RA, Zimmer AM, Pavel DG, et al. Technical parameters associated with miniaturized chromatography systems. *J Nucl Med Technol* 1979;7:19–22.