Rapid Miniaturized Chromatography for Technetium-99m-Tetrofosmin

B.F. McKay, A.M. Zimmer and S.M. Spies

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

The purpose of this investigation was to develop and evaluate a new and rapid miniaturized chromatography system that would accurately assess the radiochemical purity of ^{99m}Tc-tetrofosmin without the problems of solvent ratios and time requirements associated with the manufacturer's recommended procedure.

Methods: The migration of the radiochemical components of ^{99m}Tc- tetrofosmin was evaluated using various chromatography media with ethyl acetate as the solvent. After optimization of the miniaturized system, radiochemical purity assessments were performed simultaneously on 23 ^{99m}Tc-tetrofosmin preparations using both recommended and miniaturized chromatography systems.

Results: A miniaturized chromatography system consisting of Whatman 1 chromatography paper with ethyl acetate was developed for the radiochemical purity assessment of ^{99m}Tc-tetrofosmin. Radiochemical purity results for ^{99m}Tc-tetrofosmin preparations were similar with both recommended and miniaturized chromatography methods, with a mean difference of 1.5%±1.2% (s.d.). Differences in radiochemical purity results between the two chromatography systems were less than 2% (20 of 23 evaluations) with most preparations.

Conclusion: Radiochemical purity results for ^{99m}Tc-tetrofosmin preparations were similar with both the manufacturer's recommended chromatography and miniaturized chromatography systems. The miniaturized chromatography system is easier to use, and the time required to perform radiochemical purity assessments is substantially reduced.

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

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Technetium-99m-tetrofosmin has recently been approved for use as a myocardial perfusion agent. After preparation of this agent, the manufacturer's recommended quality control procedure can be used to determine the radiochemical purity by a single-strip chromatography system (*1*). The recommended procedure is time-consuming (25–35 min) and the results are dependent on the exact ratios of solvents used. Miniaturizing this recommended chromatography system shortened the time but did not alleviate the problem associated with precise solvent ratios (*2*). Our laboratory has developed a new and rapid miniaturized chromatography system to evaluate the radiochemical purity of ^{99m}Tc-tetrofosmin.

MATERIALS AND METHODS

The manufacturer's recommended chromatography system uses ITLC-SG (Gelman Instruments, Ann Arbor, MI) chromatography paper cut into a 2 × 20-cm strip and dichloromethane:acetone (65:35) as the developing solvent. In this system, free [^{99m}Tc]pertechnetate migrates with the solvent front (R_f = 1.0), ^{99m}Tc-tetrofosmin migrates with an R_f value of 0.5 and hydrolyzed reduced ^{99m}Tc remains at the origin (R_f = 0.0). The activity distribution of a typical chromatography strip of ^{99m}Tc-tetrofosmin preparation is shown in Figure 1. The chromatography strip is marked at the origin line, the solvent front line and two cut lines at R_f values of 0.2 and 0.8,

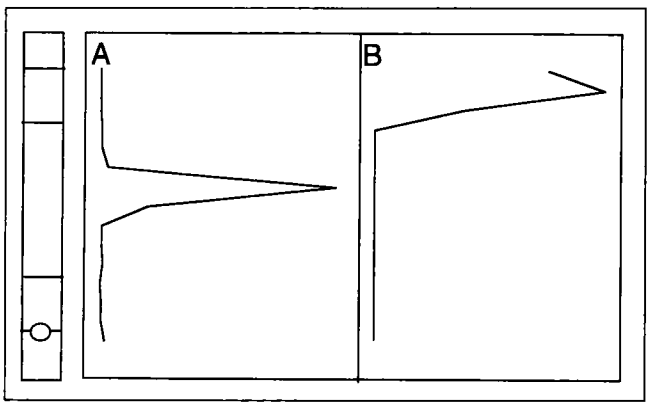

FIGURE 1. Chromatographic evaluation of (A) ^{99m}Tc-tetrofosmin and (B) [^{99m}Tc]pertechnetate using the manufacturer's recommended chromatography system. The origin is at the bottom and the solvent front is on the top.

For correspondence or reprints contact: Bernie McKay, CNMT, Department of Nuclear Medicine, Northwestern Memorial Hospital, 250 E. Superior St., Chicago, IL 60611.

TABLE 1
Migration of Technetium-99m-Tetrofosmin in Various Chromatography Media with Ethyl Acetate as the Solvent

Chromatography media	Solvent system	^{99m}Tc-tetrofosmin migration (R_f value)
Whatman 31ET	Ethyl acetate	0.3
Gelman Saturation Pads	Ethyl acetate	0.5
Whatman 17	Ethyl acetate	0.3
Whatman 4	Ethyl acetate	0.6
Whatman 3	Ethyl acetate	0.6
Whatman 1	Ethyl acetate	0.9
Whatman 2	Ethyl acetate	0.9
Whatman 20	Ethyl acetate	1.0

respectively. After solvent development, the strip is cut into the three sections. The lower section contains the hydrolyzed reduced ^{99m}Tc component, the middle section ^{99m}Tc-tetrofosmin and the upper section free [^{99m}Tc]pertechnetate.

Our laboratory initially investigated the migration of ^{99m}Tc-tetrofosmin in various chromatography media using ethyl acetate as the solvent system. Technetium-99m-tetrofosmin was spotted on 1 × 10-cm chromatography media, including Gelman saturation pads and Whatman chromatography papers No. 1, 2, 3, 4, 17, 20 and 31ET. Immediately after spotting, the chromatography strips were placed in ethyl acetate until the solvent migrated to the solvent front line. Chromatography strips were then removed, cut into 0.5-cm sections and each section counted for activity using a NaI well detector interfaced to a multichannel analyzer. The migration of ^{99m}Tc-tetrofosmin in various chromatography media is shown in Table 1. Results are listed in order from the most rapid (Whatman 31ET) to the slowest (Whatman 20) solvent migration times. From these results, Whatman 1 chromatography paper was chosen as the optimal chromatography media for use with ethyl acetate.

The new miniaturized chromatography procedure developed by our laboratory is shown in Table 2. This new procedure utilizes Whatman 1 chromatography paper cut into 0.6 × 6-cm strips with the origin and solvent front drawn 1.0 and 0.5 cm from the bottom and the top of the strip, respectively. All lines are drawn using a pencil. The dimensions and the appropriate cut line for the miniaturized strips are depicted in Figure 2. The strip is developed in a single solvent, ethyl acetate. By using this procedure, multiple ^{99m}Tc-tetrofosmin and [^{99m}Tc]pertechnetate preparations were spotted on the miniaturized strips, as outlined above, and placed in ethyl acetate until the solvent migrated to the solvent front. Chromatography strips were then removed, cut into 0.5-cm sections and each section counted for activity as outlined. From this data, chromatography strip activity distribution curves were generated.

The radiochemical purity of 23 ^{99m}Tc-tetrofosmin preparations were evaluated simultaneously using both the manufacturer's recommended chromatography system and the new

TABLE 2
Miniaturized Chromatography Procedure for Technetium-99m-Tetrofosmin

1. Place approximately 0.8–1.0 ml of ethyl acetate in a clean empty 10-ml serum vial.
2. Spot the radiopharmaceutical at the origin line using a 1-ml syringe with either a 25- or 27-gauge needle.
3. Immediately place the strip in the solvent and allow the solvent to migrate to the solvent front line. This will take approximately 2–3 min.
4. Remove the strip and cut at cut line into two sections: Section 1 (origin) and Section 2 (solvent front).
5. Count each section using an appropriate counting system.
6. Calculate the labeling efficiency as follows:

$$\% \text{ Free } [^{99m}\text{Tc}] \text{ pertechnetate} = \left[\frac{(\text{Net cts Section 1})}{(\text{Net cts Section 1}) + (\text{Net cts Section 2})}\right] \times 100$$

$$\% \text{ Bound radiopharmaceutical} = \left[\frac{(\text{Net cts Section 2})}{(\text{Net cts Section 1}) + (\text{Net cts Section 2})}\right] \times 100$$

miniaturized chromatography system, outlined in Table 2, in triplicate. The developing chambers were prepared with the required solvent for each system. Four milliliters dichloromethane:acetone (65:35) were placed in a graduated cylinder for use with the manufacturer's recommended ITLC-SC chromatography strip while approximately 1.0 ml of ethyl acetate was placed in a clean 10-ml serum vial for use with the miniaturized Whatman 1 chromatography strip. The strips were spotted at the origin line using a 1-ml syringe with either a 25- or 27-gauge needle and then immediately placed in the

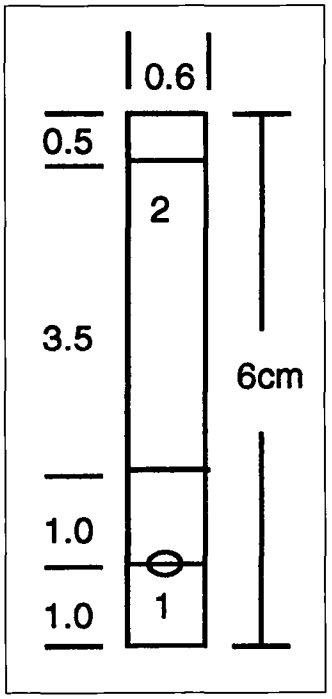

FIGURE 2. Dimensions of a miniaturized Whatman 1 chromatography strip with origin (1 cm from bottom), cut line (1 cm from origin) and solvent front (0.5 cm from top) drawn in pencil.

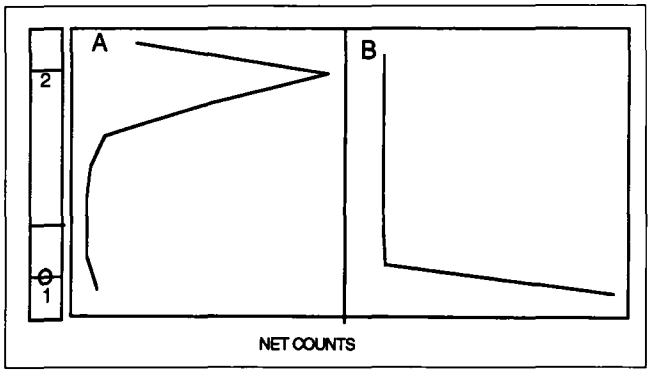

FIGURE 3. Chromatographic evaluation of (A) ^{99m}Tc-tetrofosmin and (B) [^{99m}Tc]pertechnetate using new miniaturized chromatography system. The origin is at the bottom and the solvent front is on the top.

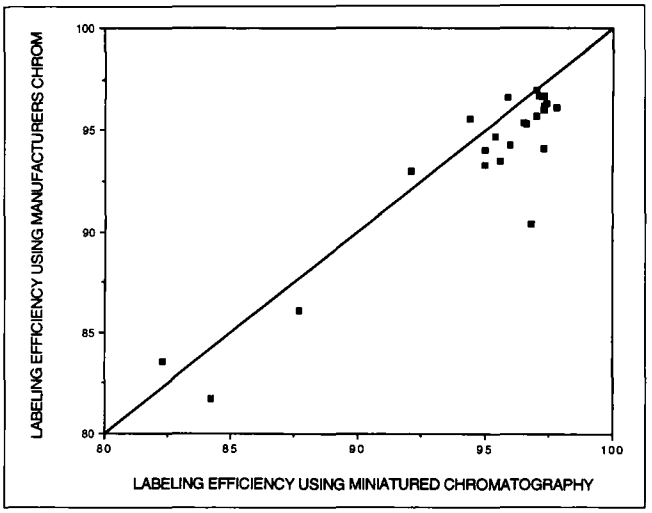

FIGURE 4. Comparison of radiochemical purity results for ^{99m}Tc-tetrofosmin using the manufacturer's recommended chromatography system versus new miniaturized chromatography system.

appropriate developing chamber. When the solvent migrated into the marked solvent front line, the strip was removed from the developing chamber and cut at the indicated cut lines. The development time required for both the recommended and miniaturized chromatography systems was noted. The sections were then counted for activity using a NaI well detector interfaced to a multichannel analyzer and the radiolabeling efficiencies were calculated. The data were then summarized by calculating mean differences in radiochemical purity and standard deviations between each specific chromatgraphy system.

RESULTS

The chromatography strip activity distribution of ^{99m}Tc-tetrofosmin and [^{99m}Tc]pertechnetate using the new miniaturized chromatography system is shown in Figure 3. Free [^{99m}Tc]pertechnetate and hydrolyzed reduced ^{99m}Tc remain at the origin in this system (R_f = 0.0). Technetium-99m-tetrofosmin showed excellent separation from both free and hydrolyzed reduced ^{99m}Tc with a R_f value of 0.9–1.0.

Radiochemical purity assessments were performed on 23 ^{99m}Tc-tetrofosmin preparations using both miniaturized and recommended chromatography systems. Results of the study are shown in Figure 4. Labeling efficiencies ranged from 81.7% to 97.8%. Similar radiochemical purity results were observed with both chromatography systems, with mean differences of 1.5% ± 1.2% s.d. Differences in radiochemical purity results between the two chromatography systems were less than 2% for 20 of 23 evaluations.

It took 30 min to develop the manufacturer's recommended strip and 3 min for the miniaturized strip, a 10-fold reduction in developing time.

DISCUSSION

The conventional quality control method used to assess radiochemical purity of ^{99m}Tc-tetrofosmin uses ITLC-SG paper (20 × 2 cm) in a solvent system of dichloromethane: acetone (65:35). With this system, it is critical that the proper solvent ratio be obtained. Any slight alteration of the solvent ratios will have a direct effect on the migration of ^{99m}Tc-tetrofosmin and the quality control results. This quality control method is also time-consuming.

Our laboratory has developed and evaluated a miniaturized chromatograpy system consisting of Whatman 1 chromatography paper (0.6 × 6 cm) with ethyl acetate to assess radiochemical purity of ^{99m}Tc-tetrofosmin. The dimensions and various line markings of the chromatography strips are shown in Figure 2. In our experience, it is extremely important that the lines on the chromatography strips (the origin, cut lines and solvent front lines) be drawn in pencil since other marking instruments can interfere with the migration and could also bind the radiopharmaceutical, resulting in inaccurate radiochemical purity assessments. In addition, the use of ethyl acetate as the solvent alleviates the problems associated with solvent mixtures.

CONCLUSION

Our results demonstrate that the new miniaturized chromatography system is effective in evaluating the radiochemical purity of ^{99m}Tc-tetrofosmin. These radiochemical purity results were available within 3 min and required no mixing of solvents, resulting in a 10-fold time savings over the manufacturer's recommended chromatography procedure.

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