Modified Preparation and Rapid Quality Control Test for Technetium-99m-Tetrofosmin

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Objective: The objectives of this study were to: modify the preparation of ^{99m}Tc-tetrofosmin by using twice the amount of $^{99m}TcO_4^-$ recommended by the manufacturer; evaluate the use of miniaturized rapid paper chromatography (MRPC) for quality control (QC) testing; and determine the in vitro stability of the modified preparation using MRPC.

Methods: Two preparations of ^{99m}Tc-tetrofosmin were made: one with 4.4–8.8 GBq (120–240 mCi) and the other with 13.9–17.6 GBq (380–480 mCi) $^{99m}TcO_4^-$, referred to as regular and modified preparations, respectively. Routine QC tests were performed using MRPC and instant thin-layer chromatography/silica-gel (ITLC/SG) systems. The preparations were injected into 58 patients. Planar and SPECT images of stress and rest studies were obtained. The technical quality of the SPECT images was graded visually by four observers. Heart-to-lung and heart-to-background ratios were calculated from the planar images.

Results: The QC testing procedure took 4.18 ± 0.15 min with MRPC and 54 ± 5.3 min with ITLC/SG systems. The percent labeling efficiency, as determined by both techniques, ranged from 95.6 ± 1.6 to 97.2% ± 0.8%. Both preparations were stable up to 6 hr after reconstitution. There was no difference between the cardiac-to-lung and cardiac-to-background ratios of the two preparations.

Conclusion: The results indicate that MRPC is a faster and effective chromatographic technique for routine QC testing of ^{99m}Tc-tetrofosmin. Doubling the amount of $^{99m}TcO_4^-$ used in preparing ^{99m}Tc-tetrofosmin did not affect its in vitro stability, its efficacious use in patients or the technical quality of the images.

Key Words: technetium-99m-tetrofosmin; quality control; preparation; image quality

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Technetium-99m 1,2-bis[bis (2-ethoxyethyl) phosphino] ethane (^{99m}Tc-tetrofosmin) is a new radiopharmaceutical used in clinical nuclear medicine practice for myocardial perfusion imaging (*1–4*). It is used also for imaging noncardiac tissues, such as thyroid, lung and breast cancers (*5–7*). The manufacturer recommends the addition of 4.4–8.8 GBq (120–240 mCi) $^{99m}TcO_4^-$ to a vial of tetrofosmin (Myoview®; Amersham Intl. plc, London, UK) (*8*). The addition of larger than specified amounts of $^{99m}TcO_4^-$ results in less personnel radiation exposure relative to making one preparation instead of multiple preparations, less variability in quality control (QC) results with fewer preparations, and more convenience. In order to use a larger amount of $^{99m}TcO_4^-$ to prepare ^{99m}Tc-tetrofosmin it is necessary to evaluate the in vitro and in vivo stability of the resulting product.

Technetium-99m-tetrofosmin, like other ^{99m}Tc preparations, probably contains $^{99m}TcO_4^-$, reduced-hydrolyzed ^{99m}Tc ($^{99m}TcO_2$) or other ^{99m}Tc-complexed species. To determine the presence of these radiochemical impurities and to quantitate them, the manufacturer recommends the use of thin-layer chromatography (TLC) which uses the Gelman instant thin-layer chromatography/silica-gel (ITLC/SG) system (20 cm × 1 cm) developed in a 35:65 v/v mixture of acetone and dichloro-methane. This recommended procedure is time consuming (48–59 min). Using this procedure, it may not be possible to check the radiochemical purity of ^{99m}Tc-tetrofosmin before its administration to a patient. Hence, the need to develop and evaluate a time-saving technique for the QC testing of ^{99m}Tc-tetrofosmin.

The objectives of this study were to: (a) modify the preparation of ^{99m}Tc-tetrofosmin by using approximately twice the recommended amount of $^{99m}TcO_4^-$; (b) evaluate the use of the miniaturized rapid paper chromatographic (MRPC) method (*9*) for the QC testing of ^{99m}Tc-tetrofosmin and compare it to the TLC system; and (c) determine the in vitro stability of the modified preparation of ^{99m}Tc-tetrofosmin using MRPC and assess its efficacious use by injecting it into patients and performing both planar and SPECT imaging.

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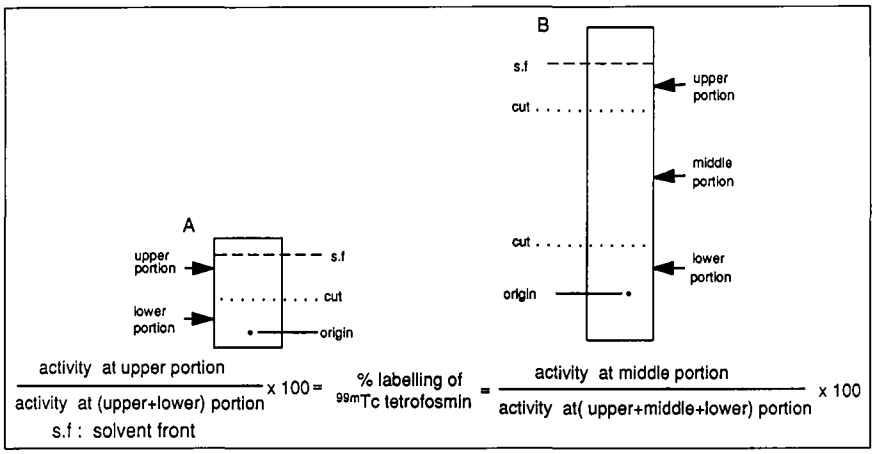

FIGURE 1. The miniaturized rapid paper chromatography (MRPC) and instant thin-layer chromatography (ITLC) strips indicating the areas to be cut. The (A) MRPC strip and the (B) ITLC strip are cut into two and three portions, respectively.

MATERIALS AND METHODS

Preparations

Two preparations were made: one according to the manufacturer's recommendation by adding 4.4–8.8 GBq (120–240 mCi) and the other by adding 13.9–17.6 GBq (380–480 mCi) $^{99m}TcO_4^-$ to a vial of Myoview®. These preparations will be referred to as the regular and modified preparations, respectively. For the radiochemical QC test, 10 preparations were made and each preparation was tested with each chromatographic method described below.

Evaluation of the Miniaturized Rapid Paper Chromatographic System

Preliminary experiments were performed to establish the R_f values of ^{99m}Tc-tetrofosmin, $^{99m}TcO_4^-$ and $^{99m}TcO_2$. The $^{99m}TcO_2$ was prepared by reducing $^{99m}TcO_4^-$ with stannous ion at pH > 5 in the absence of a complexing or chelating agent. Approximately 10 μl of ^{99m}Tc-tetrofosmin, $^{99m}TcO_4^-$ or $^{99m}TcO_2$ were placed 1 cm above the lower end of 12-cm Whatman No. 3 MM paper strips and developed up to 10 cm by the ascending method in ethylacetate under atmospheric conditions. The strips were air-dried, cut into 0.5-cm pieces and counted. The R_f value was determined by the standard equation:

$$R_f = \frac{\text{Distance migrated by solute}}{\text{Distance migrated by solvent}}. \quad \text{Eq. 1}$$

For radiochemical QC testing of ^{99m}Tc-tetrofosmin, 10 μl of the preparation were placed 1 cm from the lower end of a Whatman 3 MM paper strip (7 cm × 0.5 cm). The strip was developed up to 6 cm in ethylacetate and air dried. Based on the R_f values of ^{99m}Tc-tetrofosmin, $^{99m}TcO_4^-$ and $^{99m}TcO_2$ established previously, the strip was cut at 3 cm from the lower end into two pieces (Fig. 1). Each piece was separately counted in a dose calibrator. Percent labeling efficiency of ^{99m}Tc-tetrofosmin was calculated as:

$$\% \ ^{99m}\text{Tc-tetrofosmin} = \frac{\text{Activity at (upper portion)}}{\text{Activity at (upper + lower) portions}} \times 100. \quad \text{Eq. 2}$$

Thin-Layer Chromatographic System

A drop of 10–20 μl ^{99m}Tc-tetrofosmin preparation was applied 3 cm from the lower end of the ITLC/SG plate (2 cm × 15 cm). The ITLC chromatographic plates were developed in a jar containing acetone:dichloromethane (35:65) as a solvent. The ITLC plates were removed from the jar when the solvent reached 10 cm from the bottom and allowed to dry. For R_f values the plates were cut into 1-cm pieces; each piece was counted separately in a dose calibrator and R_f values were determined as in Equation 1. For the radiochemical QC test, the ITLC plates were cut at 2 cm and 7 cm from the lower end into three pieces (Fig. 1B). The radioactivity of each portion was counted separately. Percent labeling efficiency of ^{99m}Tc-tetrofosmin was calculated as:

$$\% \ ^{99m}\text{Tc-tetrofosmin} = \frac{\text{Activity at (middle portion)}}{\text{Activity at (upper + middle + lower) portions}} \times 100. \quad \text{Eq. 3}$$

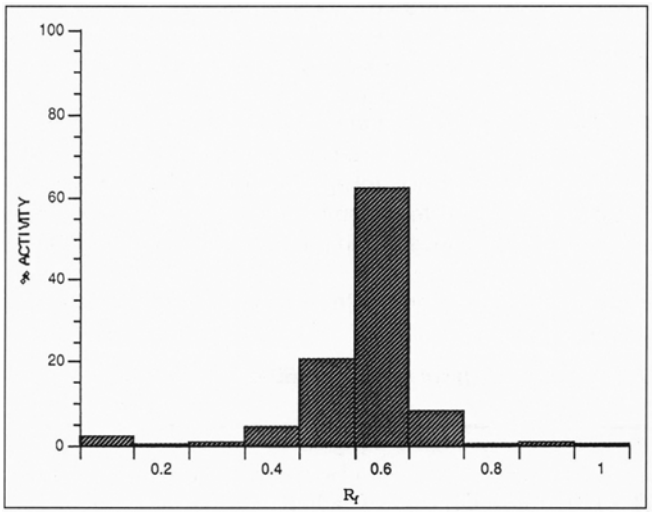

FIGURE 2. The chromatogram of ^{99m}Tc-tetrofosmin using the miniaturized rapid paper chromatography/ethylacetate system. Note the ^{99m}Tc-tetrofosmin can be separated easily from both $^{99m}TcO_2$ and $^{99m}TcO_4^-$ whose R_f value is 0.00.

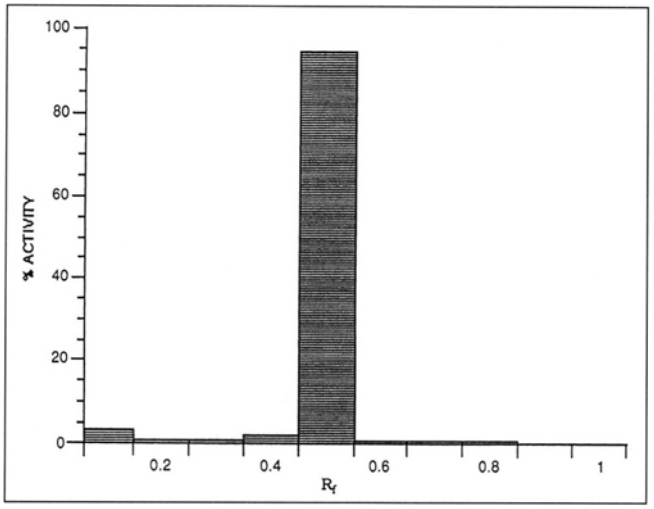

FIGURE 3. The chromatogram of ^{99m}Tc-tetrofosmin using the ITLC-SG/acetone:CH_2Cl_2 system. The ^{99m}Tc-tetrofosmin can be separated easily from both $^{99m}TcO_2$ and $^{99m}TcO_4$ whose R_f values are 0.00 and 0.9–1.0, respectively.

Evaluation of Stability and Efficacy of the Preparations

The in vitro stability of and efficacy of both the regular and modified ^{99m}Tc-tetrofosmin preparations were studied and compared to each other. The in vitro stability was studied using the previously described MRPC technique while planar and SPECT imaging were used to study the efficacious use of both preparations after administration to patients.

For the imaging, 58 patients with known or suspected coronary artery disease referred to the department for stress and rest myocardial perfusion were studied. Both planar and SPECT studies were performed in 16 patients while only SPECT studies were performed in the remaining 42 patients. Each patient was injected with 740 MBq (20 mCi) ^{99m}Tc-tetrofosmin. All patients received both the regular and the modified preparations. For each patient, the preparations were switched during the stress or rest study. The imaging was performed with a rectangular field-of-view gamma camera, using a low-energy general-purpose collimator, interphased with a computer. The planar images were acquired in the anterior projection for 2 min. The SPECT acquisition was performed in 180° (140° LAO to 40° RAO) using a 64 × 64 matrix. A Hanning filter set of 0.8 cutoff frequency was used for the tomographic reconstruction. Four observers visually graded the image quality for technical acceptance.

The planar images were used for quantitation. Fixed sizes of regions of interest (ROIs) were drawn at the anterior, apex and inferior walls of the left ventricle. The same size ROI also was drawn for lung and background sites. Cardiac-to-lung and cardiac-to-background ratios were calculated. The ratios of either stress or rest studies obtained with the regular preparation of ^{99m}Tc-tetrofosmin were compared to the respective values obtained with the modified preparation.

TABLE 1
R_f Values of Technetium-99m Species by the Chromatographic System Used

Technetium-99m species	Rf values: 3 MM/ethyl acetate (MRPC)	Rf values: ITLC-SG/Acetone: CH_2Cl_2 (35:65)
^{99m}Tc-tetrofosmin	0.4–0.7	0.4–0.5
$^{99m}TcO_4^-$	0.0	0.9–1.0
$^{99m}TcO_2$	0.0	0.0

TABLE 2
Radiochemical Purity Determination of Regular and Modified Preparations of Technetium-99m Tetrofosmin

Chromatographic system	Regular (n = 10)	Modified (n = 10)
Whatman 3 MM paper/ethylacetate	97.2 ± 0.8	97.50 ± 0.5
ITLC-SG/Acetone: CH_2Cl_2 (35:65)	95.6 ± 1.6	96.8 ± 0.2

RESULTS

The chromatograms of ^{99m}Tc-tetrofosmin, using the two chromatographic techniques, are shown in Figures 2 and 3. The corresponding R_f values, including those of $^{99m}TcO_4^-$ and $^{99m}TcO_2$, are summarized in Table 1. With the MRPC system (Whatman 3 MM/ethylacetate), the R_f range of ^{99m}Tc-tetrofosmin was 0.4–0.7 while that of both $^{99m}TcO_4^-$ and $^{99m}TcO_2$ were 0.0. With the manufacturer's system (ITLC/acetone: CH_2Cl_2), the R_f range of ^{99m}Tc-tetrofosmin, $^{99m}TcO_4^-$ and $^{99m}TcO_2$ were 0.4–0.5, 0.9–1.0 and 0.0, respectively (Table 1).

The percent labeling efficiency, as determined by the two techniques for both regular and modified preparations of the ^{99m}Tc-tetrofosmin, ranged from 95.6 ± 1.6 to 97.5% ± 0.5% (Table 2). The times spent in performing the QC testing of the ^{99m}Tc-tetrofosmin preparations were 4.18 ± 0.15 min for the MRPC and 54 ± 5.3 min for ITLC-SG (Table 3). Both the regular and modified preparations of ^{99m}Tc-tetrofosmin were stable at room temperature up to 6 hr after preparation (Table 4).

Example SPECT images are given in Figure 4. Figures 4A and B were obtained from the regular preparation, while Figures 4C and D were generated from the modified preparation. Figures 4A and C are stress images showing inferolateral defects that filled in on the rest images (Fig. 4B, D). The

TABLE 3
Time Required to Determine Radiochemical Purity Using the Two Chromatographic Systems

Chromatographic system	n	Time (min)
3MM paper/ethylacetate (MRPC)	10	4.18 ± 0.15
ITLC-SG/Acetone: CH_2Cl_2	10	54 ± 5.3

TABLE 4
In Vitro Stability with Time Using the MRPC System

Postpreparation time	Percent labeling efficiency	
	Regular (n = 3)	Modified (n = 3)
15 min	95.70 ± 0.9	96.06 ± 1.0
30 min	97.14 ± 1.1	96.30 ± 0.9
1 1/2 hr	96.75 ± 0.8	97.58 ± 1.5
6 hr	95.53 ± 1.2	93.10 ± 1.3

technically acceptable images were 89.9% ± 2.2% and 91.4% ± 1.5% for the modified and regular preparations of ^{99m}Tc-tetrofosmin, respectively. The p-value was 0.2 indicating that there was no significant difference in the quality of the images obtained from both preparations.

The cardiac-to-lung and cardiac-to-background ratios during stress and rest studies are shown in Tables 5 and 6. The p-values, which ranged from 0.1–0.5., indicate no statistical difference between the modified and regular preparations of ^{99m}Tc-tetrofosmin.

DISCUSSION

To ascertain the chemical and radiochemical purity of a compound, the R_f value should be less than 1 but greater than 0 in both paper and thin-layer chromatography. Both the MRPC and ITLC-SG sytems satisfied this condition. The R_f range of ^{99m}Tc-tetrofosmin was 0.4–0.7 with the MRPC and 0.4–0.5 with the ITLC-SG system (Table 1). The percent labeling efficiency can be determined effectively by both systems (Table 2). However, when using the ITLC-SG/acetone:CH_2Cl_2 more than 50 min is required to complete the procedure (Table 3), while the MRPC procedure can be completed within 4 min. The results confirm that the manufacturer's recommended procedure is more time consuming and it is not practical to use this technique for QC testing of ^{99m}Tc-tetrofosmin before administration to a patient. It is important to point out that one of the factors that influences the biological fate of administered radiopharmaceuticals is the quality of the preparation and it is advisable to determine radiochemical purity before administration (*10*).

Doubling the amount of $^{99m}TcO_4^-$ to 16.65 GBq (450 mCi) for preparing ^{99m}Tc-tetrofosmin did not affect its in vitro stability. The preparations were stable at room temperature up to 6 hr (Table 4). The image quality of the myocardium derived from both preparations was technically acceptable as judged by four observers (Fig. 4).

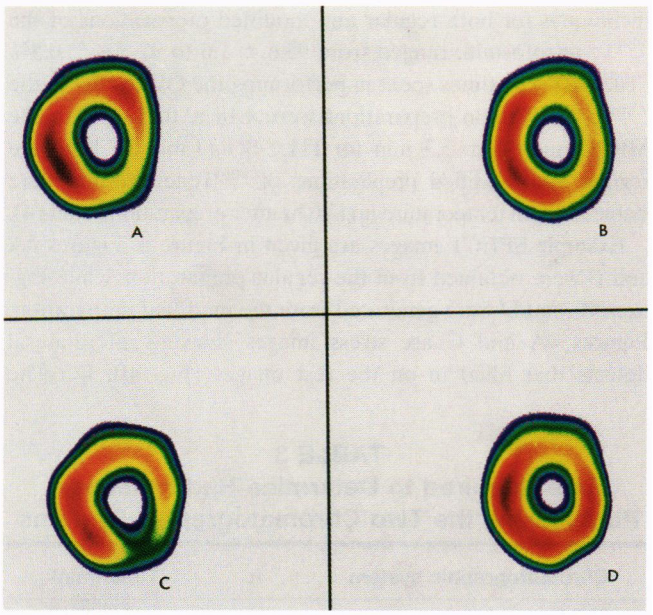

FIGURE 4. The SPECT images generated from both (A, B) regular and (C, D) modified preparations. A and C are stress images showing inferolatral defects that filled in on the rest images (B, D).

TABLE 5
Planar Heart-to-Lung Ratios

Regions	Stress studies: Preparations		
	Regular (n = 8)	Modified (n = 8)	p-value
Anterior	2.70 ± 0.57	2.59 ± 0.59	> 0.5
Apex	2.89 ± 0.59	2.95 ± 0.39	> 0.5
Inferior	2.76 ± 0.49	2.85 ± 0.63	> 0.5

Regions	Rest studies: Preparations		
	Regular (n = 8)	Modified (n = 8)	p-value
Anterior	2.39 ± 0.60	2.69 ± 0.49	> 0.2
Apex	2.60 ± 0.35	2.68 ± 0.43	> 0.5
Inferior	2.51 ± 0.44	2.77 ± 0.52	> 0.2

TABLE 6
Planar Heart-to-Background Ratios

Regions	Stress studies: Preparations		
	Regular (n = 8)	Modified (n = 8)	p-value
Anterior	5.26 ± 1.03	5.81 ± 1.91	> 0.5
Apex	5.68 ± 0.99	6.95 ± 1.64	> 0.2
Inferior	5.41 ± 1.03	6.35 ± 1.81	> 0.2

Regions	Rest studies: Preparations		
	Regular (n = 8)	Modified (n = 8)	p-value
Anterior	5.62 ± 1.22	5.58 ± 1.25	> 0.5
Apex	6.17 ± 0.85	5.45 ± 1.10	> 0.1
Inferior	5.95 ± 1.11	5.63 ± 1.28	> 0.5

CONCLUSION

The results indicate that MRPC is a faster yet effective chromatographic technique for routine QC testing of ^{99m}Tc-tetrofosmin preparations. Doubling the amount of $^{99m}TcO_4$ used in preparing ^{99m}Tc-tetrofosmin did not compromise the image quality.

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