



Evaluation of Tetrofosmin Cold Kit Fractionation Using Alternative New Method of Quality Control for Testing Radiochemical Purity

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Abstract

Objective The aim of this study is to establish a method for the fractionation of tetrofosmin cold kit under different storage conditions and to optimize an alternate chromatography method from the reference method to test radiochemical purity (RCP).

Materials and Methods Tetrofosmin cold kit vial was fractionated aseptically in six equal fractions and stored in vials and syringes. To test the stability of the reconstituted solution for a longer duration, the mother vials and syringes were stored at two different temperatures, that is, at 4°C and at –20°C till further used. Radiolabeling of fractionated tetrofosmin was performed as per the standard labeling protocol. Radionuclide purity, radioassay, and pH were tested. Radiolabeling efficiency and RCP were determined by paper chromatography.

Results Radionuclide purity of eluate was greater than 99.9%. The pH of technetium-99m (Tc-99m) eluate and Tc-99m tetrofosmin was between 4.5–7.5 and 7.5–9.5, respectively. The deviation in the radioactivity during all measurements was less than 1%. The kits fractionated in glass vials resulted in higher radiolabeling yield and RCP as compared with kits fractionated in syringes. The RCP of glass vial versus syringe was observed to be greater than 95 versus 90% and 95 versus 80% at –20°C and 4°C, respectively.

Conclusion Tetrofosmin kit can be used in a cost-effective manner by fractionation. One tetrofosmin vial can be used in six fractions for up to 15 days when stored at –20°C and 4°C freezer temperature. The alternative method to check the RCP of Tc-99m tetrofosmin is safer and less time consuming as compared with the reference method.

Keywords

- ▶ fractionation
- ▶ radiochemical purity
- ▶ radionuclide purity
- ▶ Tc-99m tetrofosmin

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Introduction

Technetium-99m (Tc-99m)-labeled tetrofosmin is widely used in myocardial perfusion and tumor imaging. Tc-99m tetrofosmin is a lipophilic cationic complex that travels inconsistently to the cell membrane and is retained in the cells due to the presence of mitochondria that indicate the presence of active cells.^{1,2}

Single cold tetrofosmin vial contains sufficient amounts of reagent for preparing multiple doses of radiopharmaceutical and is wasted when preparing one or two doses with a single tetrofosmin vial. Fractionation of tetrofosmin cold kits can be used as a cost-effective method if done with proper technique. So, this fractionation technique can be practiced in low-volume departments where patient load is not very high. The standard method for performing quality control to check the radiochemical purity (RCP) of Tc-99m tetrofosmin is thin layer chromatography (TLC) using acetone: dichloromethane (35:65) as mobile phase and silica gel as the stationary phase. However, this method uses toxic substances and is very time-consuming too. Various authors are performing different studies to establish an alternate method to check the RCP that would be time saving and safer in comparison to the reference method.³⁻⁷ The aim of present study is to establish a method for the possibility of fractionation of tetrofosmin (Myoview) cold kit for longer time periods under different storage conditions and to develop an easy and quick alternate method to the reference method for testing RCP of Tc-99m tetrofosmin.

Materials and Methods

Commercially available tetrofosmin kit was procured from GE Healthcare with the trade name Myoview. The 10 mL glass container contains nonprogenic and sterile, lyophilized powder of 0.23 mg tetrofosmin, 0.03 mg stannous chloride dehydrate, 1.8 mg sodium hydrogen carbonate, 1 mg sodium D gluconate, and 0.032 mg disodium sulphosalicylate. The lyophilized powder is sealed under nitrogen fixation by rubber and aluminum insulation. The tetrofosmin vial contains no preservative or disinfectant.

Tc-99m pertechnetate is a widely used radioisotope for nuclear medicine procedures. The eluted Tc-99m was used for radiolabeling with tetrofosmin. Before radiolabeling of tetrofosmin with Tc-99m, the tetrofosmin cold kit (Myoview) vial was fractionated into six equal fractions aseptically inside the laminar air flow, applying two different modes of fractionation, that is, fractionation in vial and fractionation in syringes. Venting needle was not inserted through the rubber septum of tetrofosmin vial containing the lyophilized powder at the time of fractionation to prevent the oxidation of content.

Fractionation in Vial

Tetrofosmin mother vial containing lyophilized powder was brought to room temperature. The content of mother vial was reconstituted in 3 mL of normal saline. For multiple use of the single reconstituted vial on different days, 0.5 mL of reconstituted tetrofosmin solution was carefully withdrawn

each time for labeling with Tc-99m. Rubber septum of mother vial containing rest of the reconstituted tetrofosmin was sealed with parafilm after each use. To test the stability of reconstituted solution for longer duration, the mother vials were stored at two different temperatures, that is, at 4°C and at -20°C till further used.

Fractionation in Syringes

The tetrofosmin mother vial containing lyophilized powder was first brought to room temperature. The content of mother vial was reconstituted in 3 mL of normal saline. For multiple use of the single reconstituted vial on different days, the content of the vial was transferred to six sterile Dispovan syringes (tagged for kit name and batch number). For making six equal fractions, 0.5 mL of reconstituted tetrofosmin solution was carefully withdrawn from the mother vial in each of six syringes. To test the stability of reconstituted solution in syringes for longer duration, all six syringes containing 0.5 mL reconstituted tetrofosmin were stored at two different temperatures, that is, at 4°C and at -20°C temperature till further used.

Radiolabeling of Fractionated Tc-99m Tetrofosmin in Vial and Syringe

For radiolabeling, reconstituted tetrofosmin mother vial/syringe was brought to room temperature. After thawing, 0.5 mL of reconstituted content was taken from the mother vial for each preparation. As all fractionated syringes contained 0.5 mL tetrofosmin content, so single syringe was used for single dose preparation after thawing. Maximum of 10 mCi (370 MBq) of freshly eluted Tc-99m was added into 0.5 mL reconstituted content. The content was mixed gently for 10 seconds to ensure complete dissolution of the content. The content was incubated at room temperature for 15 minutes before subjecting to quality control.

Quality Control of Labeled Product

Radionuclide purity, pH radioassay, radiolabeling efficiency, and RCP were tested. Microbial QC tests were not performed due to limited resources; however, all necessary precautions were taken to maintain the sterility and aseptic conditions in the laminar air flow during the fractionation process. For radionuclide purity, Moly assay test was performed by already reported method.⁸ The vial containing eluent (Tc-99m) was placed in the Moly assay canister with enough lead thickness (6 mm thickness) to absorb the low energy gamma rays of Tc-99m, that is, 140 keV. The canister allows detection of the high-energy gamma rays of Mo-99, that is, 740 keV by dose calibrator in the setting of Mo-99. The pH of Tc-99m eluate and Tc-99m-labeled tetrofosmin was measured qualitatively using pH strips. For radioassay, the Tc-99m pertechnetate and Tc-99m-labeled tetrofosmin activity was measured using a properly calibrated dose calibrator (CAPINTEC, CRC 25 R) in Tc-99m window before injecting to the patient.

Radiolabeling Efficiency and Radiochemical Purity Tc-99m Tetrofosmin

The radiolabeling efficiency and RCP of labeled product were determined with TLC. Whatman paper 3 was used as a

stationary phase and two different mobile phase acetone and saline were used. Free Tc-99m moved with the solvent front in both acetone and saline and had R_f value 1. The hydrolyzed Tc-99m remained at base and had R_f value 0 in both the solvents. To rule out the presence of hydrolyzed Tc-99m, acetone was used. In acetone solution labeled Tc-99m tetrofosmin moved with solvent front (R_f : 0.8–1.0) that indicated absence of hydrolyzed Tc-99m. To rule out the presence of free Tc-99m 0.9% saline was used. In saline, the radiolabeled complex remained at the base of the strip (R_f : 0) that indicated absence of free Tc-99m. The run chromatography strips were read by TLC scanner (TLC-204, Comecer) equipped with BGO Detector.

Method for Thin Layer Chromatography

Acetone and normal saline were poured into two test tube to a depth of 1 cm and acetone test tube was covered to allow the solvent vapor to equilibrate. A 12-cm strip of Whatman paper 3 was marked with a pencil line at 1 cm from the bottom and at 10 cm from the pencil line. The pencil line at 1 cm indicated the origin where the sample was applied. Prepared injection of radio complex 4 to 5 μ L was applied at the origin of the strip. Care was taken to not allow the spot to get dry. Strip was placed in the test tube immediately and covered. It was ensured that the strip was not adhering to the walls of the tank and sample spot was not immersed in the mobile phase. When the solvent reached the upper mark line, the strip was removed and allowed to dry. Strip was read under the TLC scanner for 60 seconds. Area under the peak was calculated to determine the radiolabeling efficiency and RCP.

Results

Based on the molybdenum breakthrough measurement, the radionuclide purity of eluate was greater than 99.9%. The pH of Tc-99m eluate and Tc-99m-labeled tetrofosmin was measured qualitatively using pH strips and observed that it was between 4.5–7.5 and 7.5–9.5, respectively. The radioactivity was measured in Tc-99m window. The deviation in the radioactivity during all measurements was less than 1%.

Radiochemical Purity of Labeled Product of Tetrofosmin Fractionated in Vials

The RCP of the radiolabeled compound, that is, Tc-99m tetrofosmin, was found to be greater than 95% from kit fractionated in vials and stored at -20°C and 4°C , when used up to 15 days. Whatman paper 3 was used as stationary phase. In acetone solution Tc-99m-labeled tetrofosmin moved with solvent front (R_f : 0.8–1.0) that indicated absence of hydrolyzed Tc. In saline the radiolabeled complex remained at the base of the strip (R_f : 0) that indicated absence of free Tc. Single peak was observed in both mobile phase indicating absence of free TcO_4^- and hydrolyzed Tc-99m complex in saline and acetone, respectively, at various temperature, that is, -20°C and 4°C temperature, as shown in **–Figs. 1–4.**

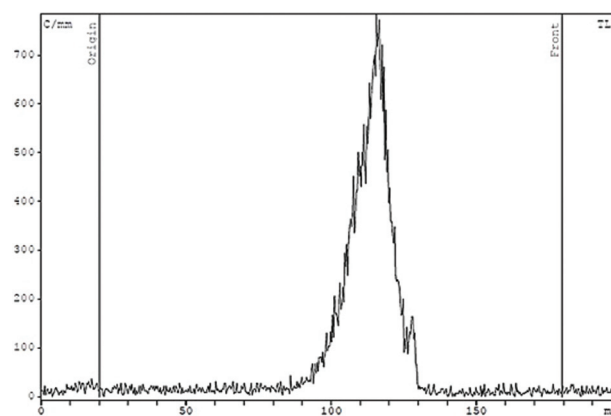


Fig. 1 Observed single peak at solvent front on thin layer chromatography done using acetone as mobile phase and Whatman paper 3 as stationary phase for technetium-99m-labeled tetrofosmin dose prepared from fractionated tetrofosmin in vial stored at -20°C temperature.

Radiochemical Purity of Labeled Product of Tetrofosmin Fractionated in Syringes

RCP for syringes containing fractionated tetrofosmin stored at -20°C was observed to be greater than 90% when used up to 15 days. Single peak was observed in both mobile phases indicating absence of free TcO_4^- and hydrolyzed Tc-99m complex in saline and acetone, respectively, at -20°C temperature as shown in **–Figs. 5 and 6.** However, RCP for syringes containing fractionated tetrofosmin stored at 4°C was observed to be greater than 80% when used up to 15 days. Two peaks were observed in both mobile phases indicating presence of free TcO_4^- and hydrolyzed Tc-99m complex in saline and acetone, respectively, as shown in **–Figs. 7 and 8.** On the basis of QC results (**–Figs. 7 and 8**), it is recommended not to store the fractionated tetrofosmin kit in syringes at 4°C because of degradation of the components resulting in reduced labeling efficiency.

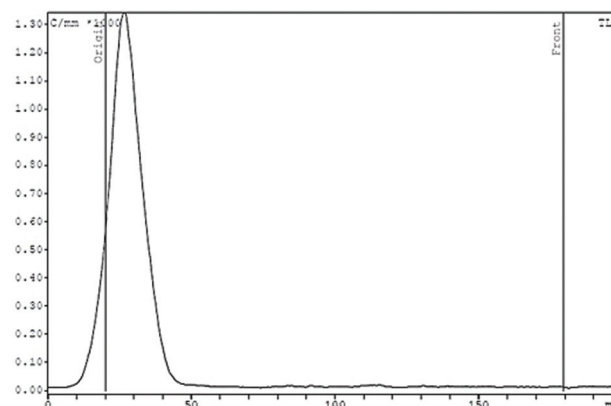


Fig. 2 Observed single peak at base of strip on thin layer chromatography done using Whatman paper 3 as stationary phase and saline as mobile phase for technetium-99m-labeled tetrofosmin dose prepared from fractionated tetrofosmin in vial stored at -20°C temperature.

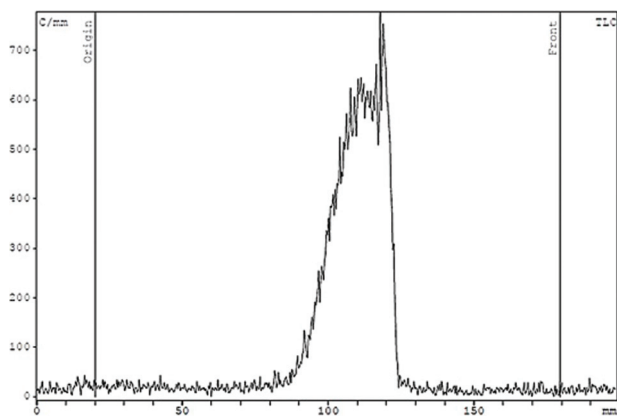


Fig. 3 Observed single peak at solvent front on thin layer chromatography done using Whatman paper 3 as stationary phase and acetone as mobile phase for technetium-99m-labeled tetrofosmin dose prepared from fractionated tetrofosmin in vial stored at 4°C temperature.

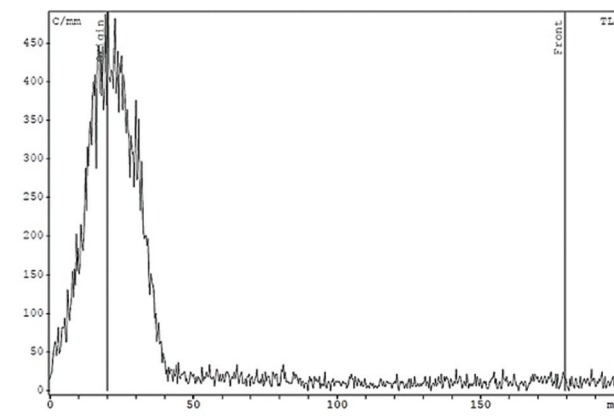


Fig. 6 Observed single peak at base of strip on thin layer chromatography done using Whatman paper 3 as stationary phase and saline as mobile phase for technetium-99m-labeled tetrofosmin dose prepared from fractionated tetrofosmin in syringe stored at -20°C temperature

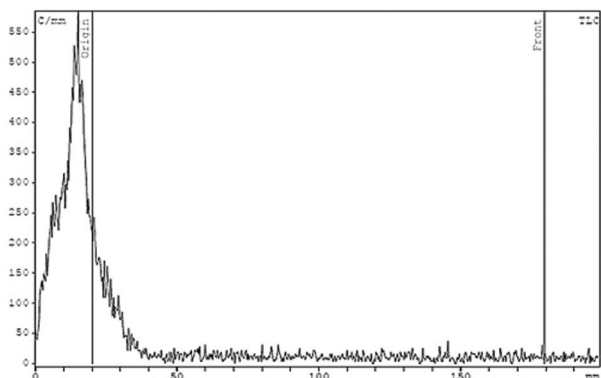


Fig. 4 Observed single peak at base of strip on thin layer chromatography done using Whatman paper 3 as stationary phase and saline as mobile phase for technetium-99m-labeled tetrofosmin dose prepared from fractionated tetrofosmin in vial stored at 4°C temperature.

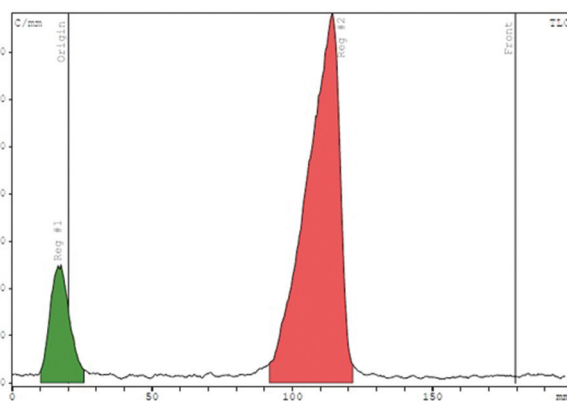


Fig. 7 Observed double peak at base of strip and solvent front on thin layer chromatography done using acetone as mobile phase and Whatman paper 3 as stationary phase for technetium-99m-labeled tetrofosmin dose prepared from fractionated tetrofosmin in syringe stored at 4°C temperature. Labeling efficiency of 84% was observed by calculating area under curve.

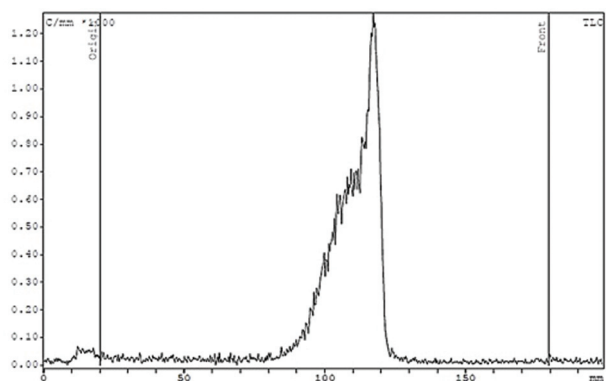


Fig. 5 Observed single peak at solvent front on thin layer chromatography done using acetone as mobile phase and Whatman paper 3 as stationary phase for technetium-99m-labeled tetrofosmin dose prepared from fractionated tetrofosmin in syringe stored at -20°C temperature.

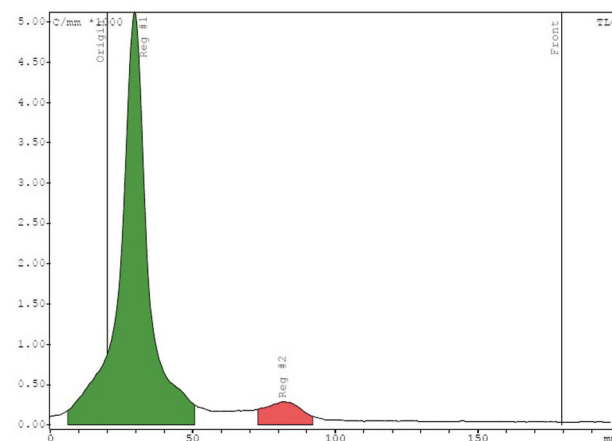


Fig. 8 Observed double peak at base of strip and solvent front on thin layer chromatography done using Whatman paper 3 as stationary phase and saline as mobile phase for technetium-99m-labeled tetrofosmin dose prepared from fractionated tetrofosmin in syringe stored at 4°C temperature. Labeling efficiency of 91.4% was observed by calculating area under curve.

Discussion

The introduction of expensive cold kits for preparing various radiopharmaceuticals has encouraged practices to aim for cost-effective use of these kits, especially in low-volume departments where the patient load is not very high. For the same reason, various authors have studied the various methods of fractionation of expensive tetrofosmin cold kit vials. The authors have studied the fractionation of tetrofosmin kit using a different combination of stationary and mobile phase to establish an easy and quick method of tetrofosmin kit fractionation.^{4-7,9}

In the present study, we have tested the possibility of fractionation of tetrofosmin (Myoview) kit to reduce the cost of the dose used per patient and proposed an easy and quick alternate method to the reference method for testing RCP of Tc-99m tetrofosmin. In comparison to the previous studies performed, present study adds the advantage of easy fractionation technique without stannous and nitrogen augmentation yielding good labeling efficiency, when done with proper technique. For storage of fractionated kits, the temperature of 4°C and -20°C attained in routinely used refrigerators is also found suitable. This will eliminate the requirement of expensive deep freezers (-80°C) recommended in other studies, hence further reducing the overall cost. In our study, we observed that all the quality control parameters indicated that the Tc-99m tetrofosmin from kits fractionated in vials was appropriate for intravenous administration. But labeling efficiency and RCP of kits fractionated in syringes stored at 4°C temperature was less and not appropriate for intravenous injection. So, fractionation of tetrofosmin kit in syringes is not recommended as per the results of present study. Tc-99m-labeled tetrofosmin dose prepared from fractionated cold kit in vials was used for routine myocardial perfusion imaging and tumor imaging in parathyroid adenomas, brain tumors, scintimammography, which proved the good labeling efficiency of these fractionated kits used for labeling as the all images were of good quality with no evidence of free technetium.

The recommended RCP test for Tc-99m with tetrofosmin is TLC with silica gel as a stationary phase and acetone: dichloromethane (35:65, v/v) as a mobile phase. However, the test is time consuming and uses a toxic substance. Various authors found the manufacturer's method inadequate and observed that solvents used in reference method need to be mixed accurately because the small variation in solvent ratio can lead to variable results.^{10,11} In our study, we proposed an alternative method to check the RCP of labeled products using Whatman paper 3 as stationary phase and acetone and saline as mobile phase. These components are easily available and used routinely in every nuclear medicine department. The Tc-99m tetrofosmin moves with the solvent front (R_f : 0.8-1) in acetone and remains at the point of spotting (R_f : 0) in case of saline. The fraction of labeled, free and hydrolyzed component can be calculated easily by determining the hydrolyzed component in acetone (R_f : 0) and free (unbound) Tc-99m (R_f : 1) in saline. The proposed

method is at par with reported TLC methods and it is safer and less time-consuming.

Conclusion

Fractionation of tetrofosmin kit can be done for multiple use of a single vial for multiple patients at different days. One tetrofosmin vial can be used in six fractions up to 15 days when stored at -20°C and 4°C freezer temperature. This feasible fractionation of tetrofosmin vial will reduce the cost of vial that will facilitate the routine use of tetrofosmin in a cost-effective manner. The alternative method is useful to check the RCP of Tc-99m-labeled chromatography using Whatman paper 3 as stationary phase, while acetone and saline as mobile phase. This method is safer and less time consuming as compared with the reference method.

Registration Number

Institutional Ethics committee (Ref No HIMS/RC/2015/1056, dated 27/07/2015).

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None.

Conflict of Interest

None.

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