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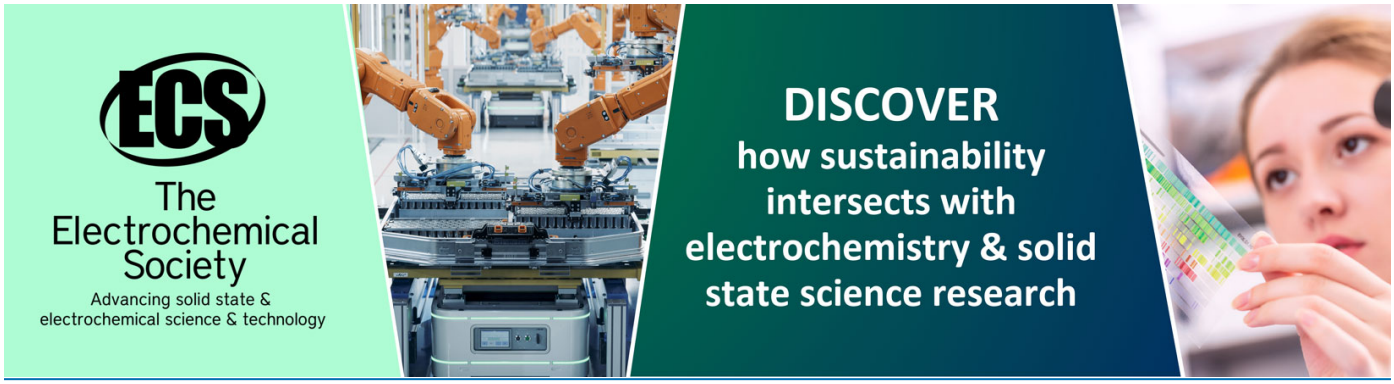

Alternative Method of Radiochemical Purity Testing for Technetium-99m Tetrofosmin

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Abstract. Tc-99m tetrofosmin is a radiopharmaceutical preparation used in nuclear medicine. Radiochemical purity as main parameter of quality should be more than 90%, and was determined using thin layer chromatography with ITLC-SG as stationary phase and mixture of acetone and dichloromethane as mobile phase. Since ITLC-SG is expensive and the solvent used is rather noxious, an alternative method is expected to replace it for routine QC use. Besides, the radiolabeling capability of Tc-99m in low radioactivity is expected to reduce the utilization of Tc-99m in QC of kits manufacturing. Experiment using SepPak C18 column and TLC system with TLC-SG and ITLC-SG were carried out, then the results were compared. Radiochemical purity measurement using TLC-SG and ITLC-SG showed 99.49 ± 0.78% and 96.17 ± 2.37% respectively with the peaks observed at Rf 0.0 and ~ 0.5 respectively, whereas that using SepPak C-18 showed RCP of 98.51 ± 0.78%. Various radioactivity of Tc-99m from 2 to 100 mCi have been used to label tetrofosmin kits and the radiolabeling quality were almost the same. It can be concluded that SepPak C18 as well as TLC method using TLC-SG can be used as alternative methods for radiochemical purity measurement of Tc-99m tetrofosmin, and low radioactivity of Tc-99m is sufficient to label tetrofosmin with high radiochemical purity.

1. Introduction

The most successful cationic, lipophilic Tc-99m radiopharmaceuticals for myocardial perfusion agents are the well-known compounds Tc-99m sestamibi (Tc-99m MIBI) and Tc-99m tetrofosmin which were developed since almost 3 decades [1]. To date Tc-99m tetrofosmin and Tc-99m MIBI have been widely used as myocardial perfusion imaging agent, but in the last decade was discovered that both Tc-99m radiopharmaceuticals display uptake in the tumor tissues, such as in breast cancer, small cell lung cancer (SCLC), parathyroid adenoma and brain tumors [2-5]. The uptake of Tc-99m MIBI and Tc-99m Tetrofosmin in this kind of tumors is supposed to be related with the over expression of tumor's multidrug resistance phenotype namely P-glycoprotein (Pgp) and multidrug resistance related proteins (MRPs) in which Tc-99m tetrofosmin is superior than Tc-99m MIBI [5]. The high uptake of Tc-99m tetrofosmin is comparable, even more useful than brain MRI which is accepted as gold standard for brain tumor imaging modality [6]. Besides, the multidrug resistance gene 1 encoding human P-glycoprotein (Pgp) is thought to play an important role in the multidrug resistance of lung cancer. Tc-99m tetrofosmin lung SPECT can accurately predict the chemotherapy response, and the same phenomena was also found in patients with malignant bone and soft tissue tumors [7-8].

Tetrofosmin kits as a pharmaceutical product must conform the requirements including the physical characteristics, biologic purity, and radiochemical purity. Biological purity includes tests for sterility and apyrogenicity, while radiochemical purity (RCP) is the percent of total radioactivity present in the desired chemical form which is a main quality parameter of radiopharmaceutical products. Unacceptable RCP can lead to localizing of the radiopharmaceutical in an unintended target, which may result in unclear image of target organ that may lead to misdiagnosing the disease. Three types of radiochemical impurities to be determined are as follows: free technetium Tc-99m pertechnetate ($Tc\text{-}99mO_4^-$), hydrolyzed-reduced Tc-99m (insoluble Tc-99m dioxide and/or Tc-99m tin colloid), and bound Tc-99m to the ligand of interest (desirable radiochemical form). These radiochemical impurities can hinder clinical interpretation by decreasing the target to non-target ratio. In clinical practice, the RCP analysis must be quick, accurate, and economical whereas in a research environment greater emphasis is placed upon accuracy, while the time and economical factors are sometimes less important [9].

All of impurities can be determined using a chromatography system, consisting of a stationary phase and a mobile (solvent) phase. The components of the mixture traveling the farthest are those that are most soluble in the solvent and least attracted to the stationary medium. The most frequently encountered procedures involve solid-phase extraction or instant thin-layer and paper chromatography. Mobile phase (solvent) moves the solute through the stationary medium affecting a separation from other solutes that are eluted earlier or later. Thin layer chromatography (TLC) is frequently used in radiochemical purity testing of radiopharmaceuticals, which uses TLC-SG as stationary phase and suitable eluants as mobile phase. The newer product of TLC-SG sheets namely ITLC-SG is widely used in research and clinical practice since it gives better performance and much faster than using TLC-SG. ITLC-SG provides superb resolution for nonpolar compounds, unfortunately, the manufacturer of ITLC-SG strips once discontinued their production, although now another manufacturer continues the production but the price is very expensive [10, 11].

According to the manufacturer's leaflet of Myoview®, QC protocol involves TLC system which uses ITLC-SG as stationary phase and mixture of acetone and dichloromethane as mobile phase [12-14]. Optimization of using shorter size of ITLC-SG strips has been reported to be as effective as that of regular size, which makes it more efficient in using this expensive consumable material [15].

Better and faster QC method was reported using SepPak cartridge as an alternative method for radiochemical purity testing of radiopharmaceuticals. Sep-Pak® cartridges are useful for chromatography since they operate on the principle of solid-phase extraction or column chromatography. The manufacturer's package inserts for Tc-99m mertiatide4 and In-111 pentetreotide recommend the Sep-Pak® C18 chromatography cartridge for the determination of radiochemical purity. This cartridge is composed of a solid, nonpolar material (sorbent) that enables the separation of the various radiochemical species of interest [9]. Another kind of SepPak cartridge, namely SepPak silica was reported to give good performance in radiochemical purity measurement of some radiopharmaceuticals including Tc-99m tetrofosmin, and can be used as an alternative for TLC method [11]. SepPak C18 cartridge was used to measure radiochemical purity of Tc-99m tetrofosmin (from Myoview® and local produced tetrofosmin kit), and found to give the same result as that of using TLC, so it can be used as an alternative method for TLC in case if there is a shortage of ITLC-SG in the market [16]. Moreover, RCP measurement using SepPak C18 is safer than using TLC, since solvents used for SepPak C18 are saline (0.9% sodium chloride solution) and ethanol, whereas the TLC system are using dichlorometane which can be classified as noxious chemicals. Labeling study of tetrofosmin kits using various radioactivity of Tc-99m of 150 mCi to 400 mCi has been reported and showed good results, this will be helpful to send a high radioactivity of Tc-99m tetrofosmin to hospitals in a ready-to-inject dosage form, to anticipate the decay during transportation since the halflife of Tc-99m is quite short (6.02 hours) [17].

Tc-99m tetrofosmin of local product (developed by PTRR-BATAN) has been previously compared with commercial product (Myoview®, GE Healthcare) in the quality of images using gamma camera (SPECT) and the results showed no significant difference between both products [18].

Labeling of tetrofosmin kits using low radioactivity of Tc-99m will be carried out, with the aim to ensure that it can perform the same quality as the one using normal and high radioactivity of Tc-99m. This information will be beneficial particularly for the manufacturer of tetrofosmin kits that needs to do quality control (QC) for each production batch. Radiochemical purity testing of final product has to be accomplished for every production batch as a requirement of Good Manufacturing Practice (GMP) for all pharmaceutical products. Therefore, the use of small amount of Tc-99m is preferable and will be more efficient.

The aim of this study is to obtain a more practical method for radiochemical purity testing of Tc-99m tetrofosmin as a part of Quality Control process in tetrofosmin kits manufacturing. Besides, the aim is to get known the minimum radioactivity of Tc-99m that can be used to label tetrofosmin kit to form Tc-99m tetrofosmin with RCP of more than 90% as stated in Pharmacopoeia as a radiopharmaceutical requirement. The use of low radioactivity of Tc-99m for routine QC activity will reduce the risk of radiation exposure to the QC personnels and will also be efficient.

2. Methods

Tetrofosmin kits of local product (PTRR BATAN)) were used to be labeled with technetium Tc-99m to form Tc-99m tetrofosmin. Tc-99m was obtained from in-house Mo-99/Tc-99m generator (PTRR-BATAN). To ensure that tetrofosmin kit was completely labeled with Tc-99m, the radiolabeled products were analysed for radiochemical purity using thin layer chromatography (TLC). The standard method of radiochemical purity testing was TLC method using ITLC-SG (Agilent) as stationary phase and mixture of acetone and dichloromethane (35:65) (both are from Merck) as mobile phase (eluant). Two alternative methods were studied, i.e. column separation using SepPak C18 cartridge (Waters) and TLC method using TLC-SG (Merck).

2.1. RCP measurement using TLC method

TLC-SG strip (1.5 cm x 10 cm) was used as stationary phase, on which 5 ul of sample was dropped at the baseline or ~ 2 cm from the strip edge, dip into mixture of acetone-dichloromethane (35:65) in a glass chamber and eluted until the solvent reached the upper edge of the strip (~1 cm from the top edge). TLC using ITLC-SG strips were used as a standard. The strips were then taken out from the chamber, air dried and measured for radioactivity by means of TLC scanner (Comecer TLC 204).

2.2. RCP measurement using SepPak C18 column/cartridge. Other separation method using SepPak C18 cartridge was studied to analyse radiochemical purity of Tc-99m tetrofosmin, in which ~ 100 ul of sample was injected into the column (cartridge) which has been previously activated with 5 ml of ethanol, water and air consecutively. The polar impurity (TcO_4^-) was eluted out of the column with 10 ml of saline, the main complex was eluted with 10 ml of ethanol-saline (4:1), and the colloid impurity (TcO_2) remained in the column. By measuring the radioactivity of the eluted fractions and the column, % radiochemical purity of Tc-99m tetrofosmin can be calculated.

2.3. Labeling capability of Tc-99m tetrofosmin with various radioactivity of Tc-99m. Labeling of Tc-99m tetrofosmin using various radioactivity of Tc-99m with the range of 2 mCi to 100 mCi was carried out and the RCP was measured using TLC (ITLC-SG as stationary phase) and SepPak C18.

3. Results and Discussion

Radiochemical purity measurement of several batches of Tc-99m tetrofosmin has been carried out using TLC system and column separation method (SepPak C18). The results were compared to the one using ITLC-SG as standard method. TLC method using TLC-SG strips performed higher radiochemical purity (RCP) than that using standard method, but since the Tc-99m complex could not migrate so the peak might be overlapped with Tc-99m colloid impurity, as can be seen at Table 1.

Table 1. Comparison between radiochemical purity analysis by TLC using TLC-SG and ITLC-SG as stationary phase

Stationary phase	Radiochemical purity (%)	Rf	
		Tc-99m Tetrofosmin	Free pertechnetate
TLC-SG strips	99.49 ± 0.78	0.0-0.1	0.9-1.0
ITLC-SG strips	96.17 ± 2.37	0.5-0.7	0.9-1.0

The identification of a radiochemical species is due to its transfer through a stationary phase via a mobile phase until it is finally separated from the other radiochemical species that are eluted earlier or later. The movement of various radiochemical species is hindered by the stationary phase's electrostatic forces (adsorption) as the solvent phase moves them forward [10].

The spotted sample volume used for all experiments was 5 µl since the volume sample spotted on TLC strips can affect the migration of the complex along the chromatogram, leading to overestimate the impurities level, this was the uniqueness of Tc-99m tetrofosmin compared to other radiopharmaceuticals [15].

The results of radiochemical purity analysis using SepPak C18 column was also similar with the one using TLC method. The advantage of using SepPak C18 column was that the separation process of the main product from its impurities was running fast, and to some experiences, the column can be reused up to 3-5 times. The series of these experiments were replicated 12 times (n=12). The results of the 3 methods can be seen in Table 2.

Table 2. Radiochemical purity measured by TLC and SepPak C18 methods

Methods	Radiochemical purity (%)
SepPak C-18	98.51 ± 0.78
TLC using TLC-SG strips	99.49 ± 0.78
TLC using ITLC-SG strips	96.17 ± 2.37

The chromatograms which used TLC-SG as stationary phase showed different peaks of Tc-99m tetrofosmin and free pertechnetate, i.e at Rf 0.0-0.1 and Rf 0.9-1.0 respectively (Figure 1), whereas the one using ITLC-SG showed the peaks at ~Rf 0.5-0.7 and Rf 0.9-1.0 respectively (Figure 2). In TLC system using TLC-SG as stationary phase the labeled compound seem to remain at the origin, and the time used to complete the elution was a bit longer, compared to the one using ITLC-SG. On the other hand, when using ITLC-SG as stationary phase the labeled tetrofosmin can move to the middle of the strip length, so it can be distinguished from the impurities which might be present at Rf 0.0 (Tc-99m colloid) and Rf 0.9-1.0 (free pertechnetate), and it conformed with the specification [12]. The advantage of using ITLC-SG compared to TLC-SG is that on ITLC-SG strip the migration of species is much faster and show better separation, but unfortunately, ITLC-SG is much expensive and made of fragile material which tends to be easily broken.

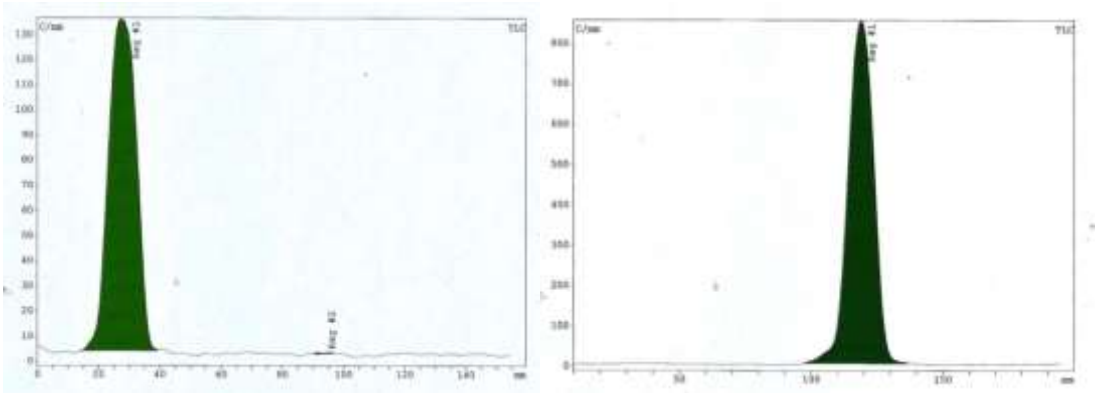

Fig 1(a) Fig 1(b)

Figure 1. Position of peaks on TLC-SG chromatogram, Fig 1(a) peak of Tc-99m tetrofosmin and Fig 1(b) peak of free pertechnetate Tc-99m

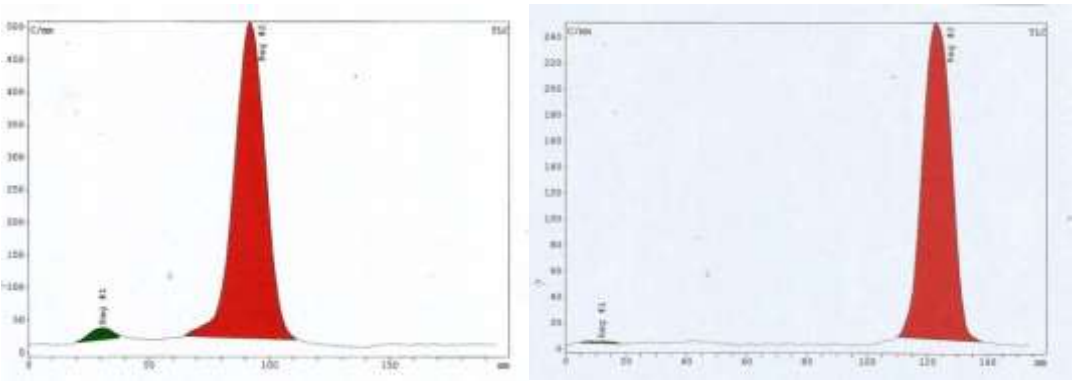

Fig 2(a) Fig 2(b)

Figure 2. Position of peaks on ITLC-SG chromatogram, Fig 2(a) peak of Tc-99m tetrofosmin and Fig 2(b) peak of free pertechnetate Tc-99m

The use of various radioactivity of Tc-99m from 2 mCi to 100 mCi in the labeling of tetrofosmin kits did not give significant difference in RCP, all of them showed RCP of more than 90% which means that the labeled compound meets the requirement of tetrofosmin kits (Figure 3). The capability of tetrofosmin kits to be labeled with low radioactivity of Tc-99m is preferable for the manufacturer of tetrofosmin kits since they do not need to consume much Tc-99m for routine QC use for every production batch.

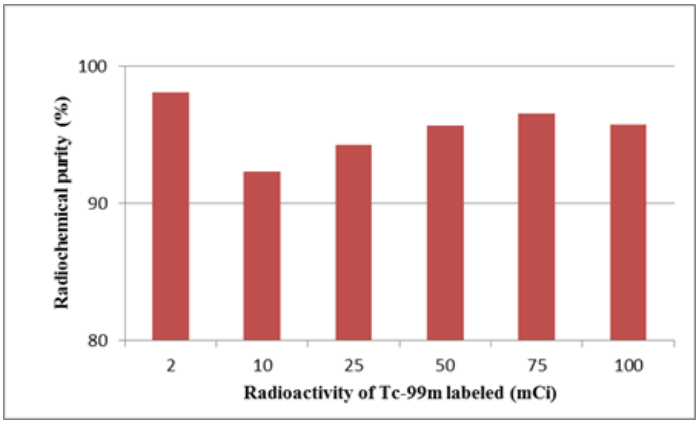

Figure 3. Labeling efficiency of Tc-99m tetrofosmin using various radioactivity of Tc-99m

4. Conclusion

RCP measurement using SepPak C18 column showed an average of 98.51 ± 0.78%, whereas using TLC method with TLC-SG as stationary phase showed RCP of 99.49 ± 0.78%, which were slightly higher than that using standard TLC method which RCP was 96.17 ± 2.37%. It is concluded that SepPak C18 can be used as an alternative method for radiochemical purity measurement of Tc-99m tetrofosmin which gives the same performance but much faster and efficient compared to standard TLC method. However, when TLC method is still preferred, an alternative TLC-SG strips can be used to substitute a much expensive ITLC-SG strips although its performance is moderate. The capability of tetrofosmin kits to be labelled with Tc-99m of only 2 mCi has been proven effective, and this will

be beneficial for the kit manufacturer since it can efficiently reduce the use of Tc-99m in implementing QC process.

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