Quality of radiochemical purity in multiple samples of various fractionated cold kits: Testing a cost & time effective technique

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Abstract

Objective: : This study was aimed to assess technical aspects of fractionation of commonly used cold kits in Nuclear Medicine. **Materials and Methods:** A total of 90 samples (30 samples each) of technetium-99m methylene diphosphonate (^{99m}Tc-MDP), ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA) and ^{99m}Tc-dimercaptosuccinic acid (DMSA III) were taken on various days. The radiochemical purity was calculated of each fraction of these cold kits by using paper chromatography. **Results:** The mean value of radiochemical purity of ^{99m}Tc -MDP, ^{99m}Tc -DTPA and ^{99m}Tc-DMSA(III) were calculated as ~ 95.12%, 91.43% and 95.68% and standard deviation (SD) were ~ 5.43, 8.36 and 3.88, respectively. Maximum time in which fractionation procedure completed i.e. time required for preparing the fraction or thawing was 10 minutes. All fractionated aliquots were between 1 and 15 days. Radiopharmaceutical bio-distribution was found to be appropriate during imaging in all samples. **Conclusion:** Fractionation of cold kits using standardised technique is a time and cost-effective method and does not deteriorate the quality of labelling in commonly used pharmaceuticals in our study. We have used fractionated aliquots up to 3 days of preparation in patients with clinically usable radiochemical purity. Deep frozen fractions can be used up to 15 days in our experience.

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Introduction

Nuclear Medicine is the branch of medical imaging in which radioactive tracers are injected to the patients to diagnose the disease. So, quality control of radioactive tracer is one of the important parameters for checking overall quality performance in nuclear medicine imaging which shows the assurance of the tracer whether it is administered to the patient or not and checking the purity of the radiopharmaceutical and radionuclide so that there is no any risk.

Nuclear Medicine is an expensive department to run and it is desirable to reduce costs wherever feasible. A single cold kit is used for a single patient which is not feasible in small centres. So, fractionation of cold kit is one of the most effective ways of utilizing a cold kit in fractions which does not compromise on quality of imaging if it is done in a standardised way.

The aim of our study was to perform, use, standardize and document the technique of fractionation of cold kits in various common single photon emission tomography (SPET) based radiopharmaceutical preparations.

Basic principles of chromatography

Paper chromatography is the chromatographic technique of separating different components on the basis of polarity. It is used for separating mixtures of compounds having similar polarity.

It has two different components: Mobile phase and Stationary phase [1, 2].

Different radiopharmaceuticals with their nature in solvents

The radiochemical purity is the fraction of the total radioactivity in the desired chemical form in the radiopharmaceutical [1]. It is checked by paper and instant thin layer chromatography but mainly paper chromatography is performed because this technique is cheaper as it requires only Whatman paper and different solvents and spectrometer for counting but it takes more time than instant thin layer chromatography (ITLC). In ITLC technique, aluminium strip coated with silica gel is used. This is read only in TLC scanner which gives the spectrum of the radiopharmaceutical and indicate the amount of free and bound fraction but does not quantitate it in the form of values.

Table 1. *Mobile and stationary phase.*

Sr No	Radio-pharmaceutical	Mobile Phase	Stationary Phase	Time duration for QC (min)	Radio chemical Yield
1	^{99m}Tc-DTPA	Saline & Acetone	Whatman paper 3 or ITLC strip	10	>95%
2	^{99m}Tc-MDP	Saline & acetone	Whatman paper 3 or ITLC strip	10	>95%
3	^{99m}Tc-DMSAIII	Acetone	Whatman paper 3 or ITLC strip	10	>95%

Table 2. *Different radiopharmaceuticals with mobile & stationary phase and their characteristics.*

Sr No	Radiopharmaceuticals	Acetone		Saline	
		Origin Front	Solvent Front	Origin Front	Solvent Front
1	^{99m}Tc-DTPA	Hydrolysed & Bound	Free	Free& Bound	Hydrolysed
2	^{99m}Tc-MDP	Hydrolysed & Bound	Free	Free& Bound	Hydrolysed
3	^{99m}Tc-DMSAIII	Bound	Free	-	

Fractionation of cold kits using standardised technique is a potential method which is practised but not documented much in existing literature [3].

Materials and Methods

Radiopharmaceutical samples prepared with fractionated vials of technetium-99m methylene diphosphonate (^{99m}Tc-MDP) and ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA) and ^{99m}Tc-dimercaptosuccinic acid (DMSA III) were taken on various days.

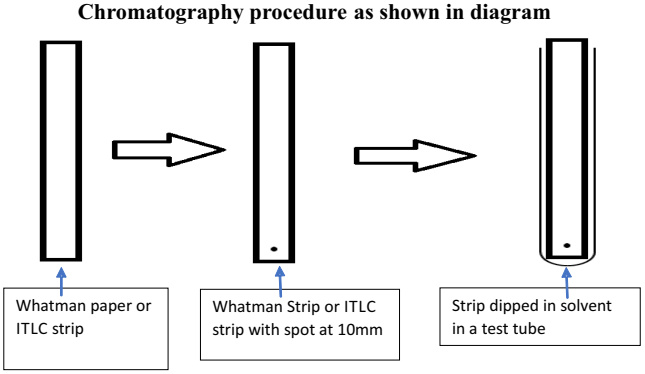
Preparation of fractionated radiopharmaceuticals

All cold kits (^{99m}Tc-MDP, ^{99m}Tc-DTPA and ^{99m}Tc-DMSA III) were fractionated in a standardised manner. One mL normal saline (0.91% NaCl) was added into each of the cold kit vial. Each vial was then divided into 3 fractions of 0.3mL each in separate vacuum vials and mixed well and put a label on these vials.

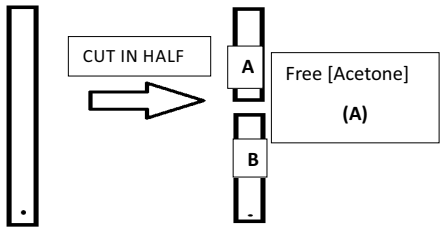
Each fraction was stored in deep freezer. The time required for performing the fractionation was also noted. On the day of use of fraction, first the fractionated vial was thaw to room temperature. The radioisotope (freshly eluted ^{99m}Tc pertechnetate) was added with a low volume in a vial and mixed well. A maximum of 2.2, 0.55 and 0.55GBq were added in MDP, DTPA and DMSA III vial fractions respectively. After mixing, the fraction was kept it for 10 minutes incubation period at room temperature. After the radiopharmaceutical preparation is completed, the quality control was performed with a sample of prepared radiopharmaceutical on the day before use.

The radiochemical purity test was done by performing paper chromatography for each of the prepared radiopharmaceuticals. In paper chromatography, stationary phase was Whatman strip III and mobile phases were acetone and saline for ^{99m}Tc-DTPA and ^{99m}Tc-MDP and for ^{99m}Tc-DMSA -III, acetone was used as solvent. In paper chromatography, a small drop of radiopharmaceutical was putting onto the Whatman strips and run in different solvents. After this, strip was taken out from the test tube and dried it. The strip was cut into two equal halves and measured into the well counter. Radiochemical purity was then calculated for each sample. Biodistribution of all samples for quality control was also studied during imaging.

Radiochemical purity (RCP) was calculated as follows:



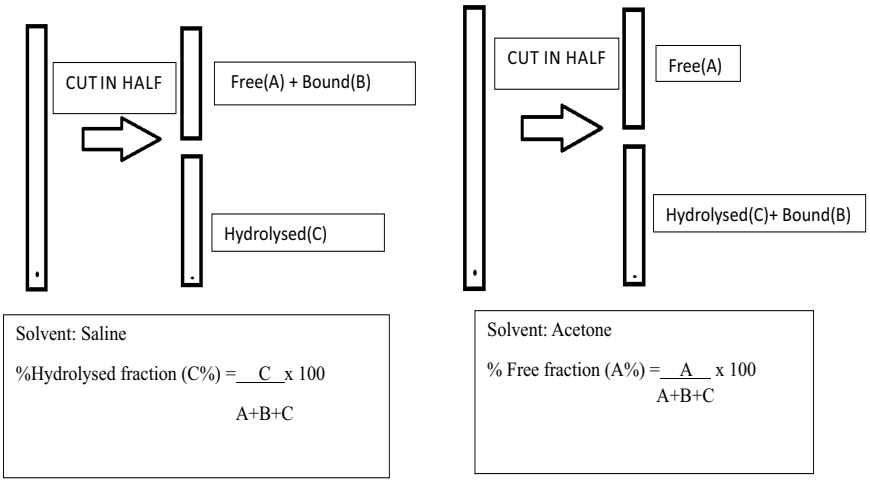
Scenario I: Free and bound form of species is present



$$\% \text{ Free fraction} = \frac{A}{A+B} \times 100$$

$$\% \text{ Bound fraction} = 100 - \% \text{ Free(A)}$$

Scenario II: Free, hydrolysed and bound form is present



% Bound fraction(B%) = 100 – A% - C%

The radiochemical purity of each of the fraction is calculated as shown [4].

Results

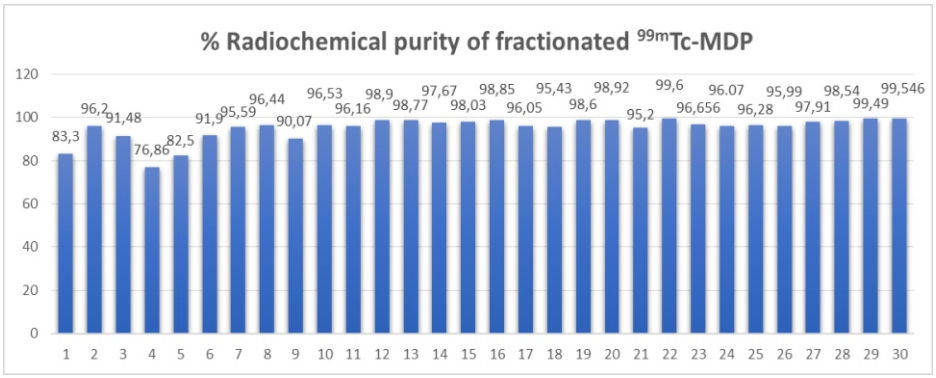
Thirty samples each of ^{99m}Tc-MDP ^{99m}Tc-DTPA and ^{99m}Tc-DMSA III were studied. The mean value of radiochemical purity i.e. labelling yield of ^{99m}Tc-MDP, ^{99m}Tc-DTPA and ^{99m}Tc - DMSA(III) were calculated as ~ 95.12%, 91.43% and 95.68%, respectively (Graph i, ii ,iii & iv). Radiopharmaceutical biodistribution was found to be appropriate during imaging in all samples (Figures 1-3). The maximum additional time in which fractionation procedure completed i.e. time required for preparing the fraction and thawing was 10 minutes. All fractionated aliquots were used within a maximum of 15 days.

Discussion

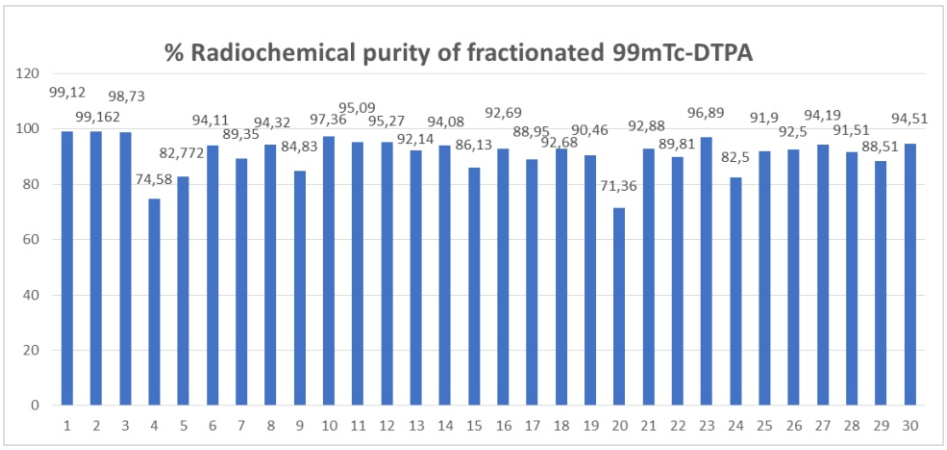
Not many studies have revealed the details of use of fractionated cold kits. Any Nuclear Medicine Unit with low patient load but with variety of patient referrals would require using aliquots for two reasons - to save costs and to be able to perform most scans daily.

Many centres utilize fractionation especially for expensive kits, however there is limited literature on this aspect [5-8]. One of the few earliest study by Thorson et al. in 1992 [6] showed effective fractionation fo the then expensive MAG 3 kits when an aliquot of five fractions was performed. Penglis et al. (2000) performed fractionation of tetrofosmin (Myoview) into 1:5 was followed by storage at -80 degrees °C which was found stable up to 3 months on reconstitution with 3.5GBq ^{99m}Tc-pertechnetate. They found no difference in biodistribution between the 1:5 fraction and the full kit, especially with respect to myocardial uptake. Myoview is an expensive kit and this is one of the few studies which documents fractionation of kits [9]. We prepared three aliquots of each kit in our study. Thus the possibility of preparing more than three can be studied.

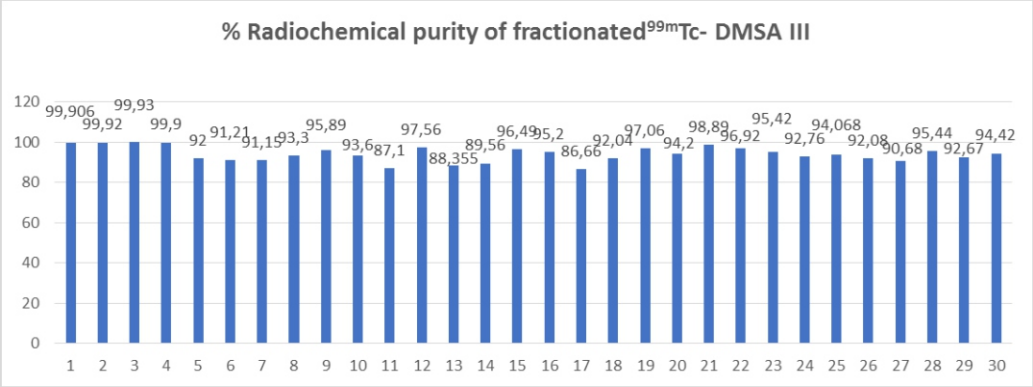
Quality, safety and radiochemical purity of radiopharmaceutical prepared with fractions must be proven so that they can be used in practice. This requires two major steps I. Standardised aliquot preparation protocol and II. Establishing quick quality control protocols, followed by incorporation into local standard operating procedures.



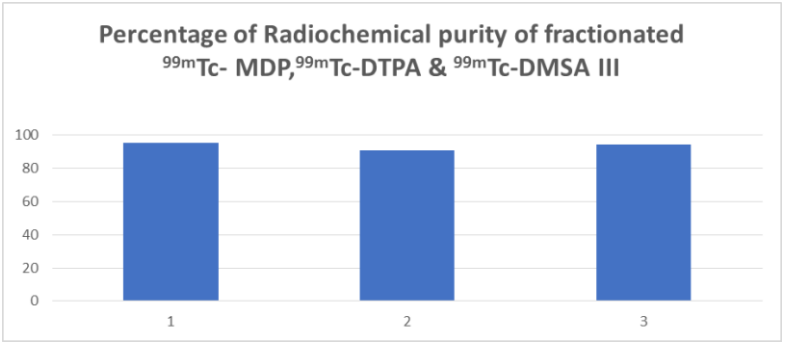
Graph (i)



Graph (ii)



Graph (iii)



Graph (iv)

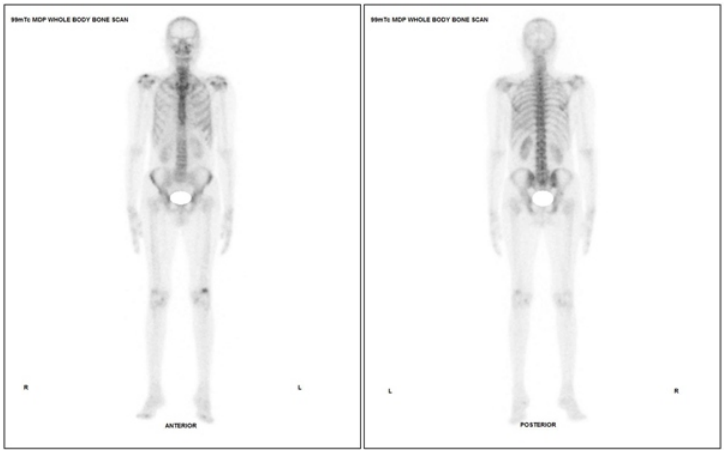


Figure 1. Biodistribution of fractionated ^{99m}Tc-MDP.

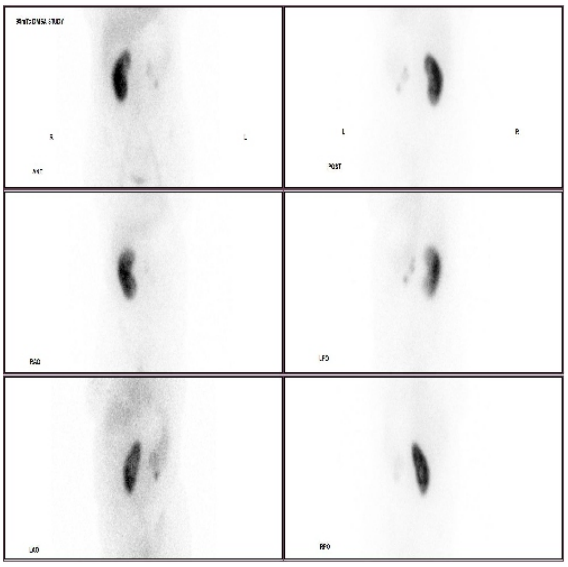


Figure 2. Biodistribution of fractionated ^{99m}Tc-DMSA(III).

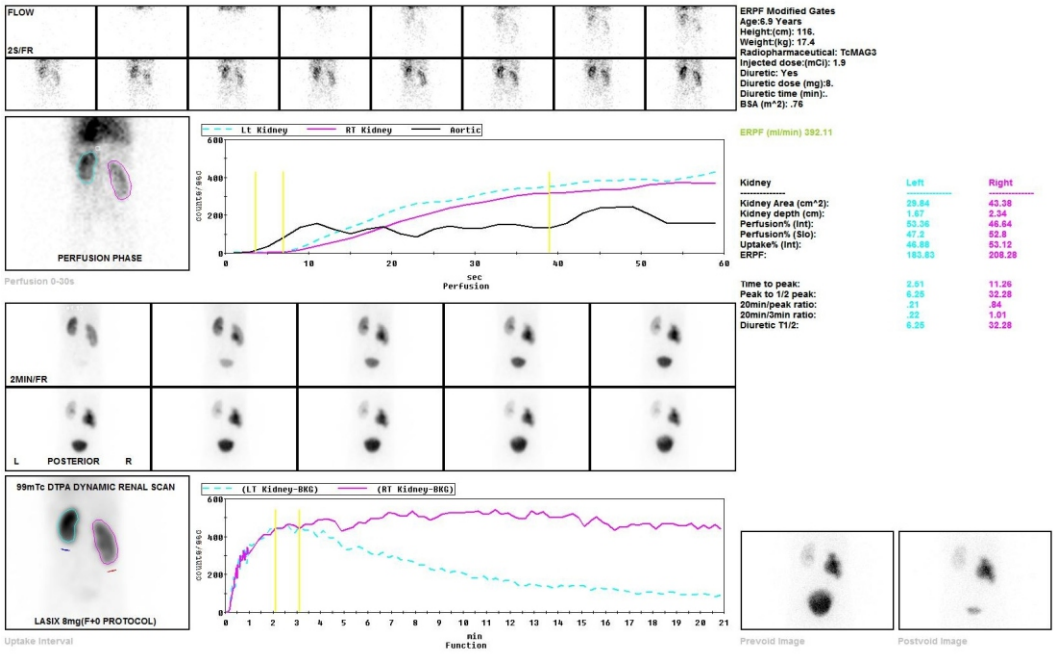


Figure 3. Biodistribution of fractionated ^{99m}Tc-DTPA.

The average radiochemical purity (RCP) by Penglis et al. (2000) was maintained at 96.5±1.6% (n=24) for 24h. In our present study, the mean value of radiochemical purity of the fractions was calculated as ~ 95.12%, 91.43% and 95.68%, respectively. In our study we found stability up to 8 hours/24 hours. We are in the process of incorporating these findings in our standard operating procedures manual.

The biodistribution of these fractions were shown as normal during the imaging with different samples in our study. Baker et al. (1999) found that fractionation of exametazime kits failed with saline only technique within one week. They noted that the addition of stannous pyrophosphate solution immediately before ^{99m}Tc-pertechnetate produced 90.1± 1.6% (n=8) lipophilic complex with a normal rate of decomposition. They suggested utilization of this technique for fractionation of exametazime kits. They also suggested this technique further for removal of restrictions on generator eluate and 'rejuvenation' of expired kits [10]. This could be prctised for older kits, however we did not perform this step in our current study.

L,L-ethylcysteinate dimer (neurolite) kits' ligand remainder has been shown to be used for up to 4 weeks when stored in deep freezer and reconstituted with up to 3.7GBq for a 0.5mL volume of ligand [11]. We have added 2.2, 0.55 and 0.55GBq were added in MDP, DTPA and DMSA III vial fractions, respectively.

We utilised all our fractions within 3 days and fractions were stored in deep freezer. The average time used for fractionation procedure in labelling was 10 minutes. Overall the time for preparing the aliquot and re-preparing the radiopharmaceutical dose with the fraction did not practically pose any time related issues like additional decay of radiopharmaceutical, delay in injection or additional loss of gamma camera time.

We found this technique to be especially helpful in centres with low patient volume but high variety of referrals. Most Nuclear Medicine procedures do not require much preparation so this allows performing the scan earlier or probably on the same day without having to club many patients for cost effectiveness.

In conclusion, fractionation of cold kits using this standardised technique is a cost-effective method and saves wastage of chemical components of kits. In our experience this process gives clinically useful labelling efficiency of studied radiopharmaceuticals. Time taken for performing aliquots and reconstitution even with quality control is feasible in routine practice. Deep frozen fractions were used up to 15 days in our study but can additionally be used up to three months as per available literature.

The authors declare that they have no conflicts of interest

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