

Quality Assessment of Tc-99m Methylene Diphosphonate (MDP) Radiopharmaceutical Prepared Using Different Cold Kit Fractionation Methods

Abstract

Purpose: Tc-99m Methylene diphosphonate (MDP) is prepared in house by labeling MDP cold kit. Each kit is a single time use vial and contains large amounts of reagent sufficient for preparing multiple doses. Therefore, several centers are adopting the practice of fractionating the MDP kit so that it can be used multiple times. The aim of the study was to evaluate the effect of kit fractionation on radiopharmaceutical property. **Materials and Methods:** The MDP kit was fractionated using two different approaches, namely, vial and syringe method. The quality of Tc-99m MDP prepared using these approaches was assessed and compared with that prepared by the conventional method. The image quality was evaluated in a total of 100 patients. **Results:** The vial and syringe fractionated Tc-99m MDP showed >95% RCP till the 4th and 2nd days of fractionation, respectively. Percentage radiochemical purity deteriorated to 83.6% and 88% on the 8th day of fractionation in the vial and syringe method, respectively. No microbial growth was observed in any of these methods till the 8th day of fractionation. The reconstituted MDP solution during all preparations was clear and colorless in appearance with pH ranging from 6.5 to 7.5. The image contrast, contrast-to-noise, and signal-to-noise ratio were statistically similar in both methods compared to the conventional method until the 2nd day of fractionation. The image quality data showed no statistical difference among images of vial and syringe fractionated MDP as compared to the conventional unfractionated Tc-99m MDP. **Conclusions:** The observations revealed that if fractionated with utmost care, both methods yield almost similar results.

Keywords: Bone scan, kit fractionation, MDP, radiopharmaceutical, sterility

Introduction

Bone scintigraphy is one of the commonly performed nuclear medicine procedures. Technetium 99m-methyl diphosphonate (Tc-99m MDP) is an ideal bone scintigraphy agent due to its high bone-to-soft tissue uptake, rapid blood clearance, and good in-vivo stability.^[1-3] Tc-99m MDP is chemisorbed on the bone tissue based on the osteoblastic activity of bony remodeling and blood flow to the tissue. The maximum amount of injected Tc-99m MDP localizes into the bone, and the rest is excreted through the kidneys. Decreased localization is seen in the areas of reduced blood flow or infarction. Diminished uptake is also seen in areas of severe destruction that can occur in some very aggressive metastasis.^[4]

Tc-99m MDP is prepared in-house by labeling MDP which is available

commercially as a cold kit. The commercially available MDP cold kits commonly contain methylene diphosphonate, stannous chloride, and gentisic acid. Methylene diphosphonate is the major component of any MDP cold kit. The stannous chloride reduces Tc-99m and enables it to form a chelate bond with the MDP carrier molecule, the gentisic acid is used as an antioxidant.^[4]

Each MDP kit is a single time use vial and contains large amounts of reagents sufficient for preparing multiple doses of a radiopharmaceutical.^[5] However, because of the nonuniform patient flow, the small hospital centers have to accept individual patients for the Tc-99m MDP study which leads to inefficient use of the kit, thereby incurring extra costs to the centers. Therefore, such centers are adopting the practice of fractionating the MDP kit so that only required amount of reagents can

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be used from the kit for single-dose preparation and rest can be saved for later use.^[5,6] The commonly practiced fractionation methods for this are vial method and syringe method. In the vial method, the pharmaceutical MDP vial (mother vial) is reconstituted in normal saline and the entire contents of the vial is distributed into multiple sterilized vials and stored at -20°C .^[2] Then, randomly selected frozen fractionated vials are later defrosted before radiolabeling.^[7] In the syringe method, the pharmaceutical MDP vial (mother vial) is reconstituted with normal saline and the required amount is withdrawn for radiolabeling in the syringe. The remaining mother vial is stored at -20°C for later use. None of these methods are scientifically validated and to the best of our knowledge, no study has been conducted for the assessment of final labeled product quality with these methods. Therefore, in the present study, we compared these two methods in terms of stability and quality of labeled compounds.

Materials and Methods

Chemicals/reagents

Mo-99–Tc-99m Generator (TCM-1 Coltech Generator, Board of Radiation and Isotope Technology, Mumbai), methylene diphosphonate (MDP) (10 mg MDP, 5 mg NaCl, 0.5 mg gentisic acid, 0.6 mg Stannous chloride) kit procured from BRIT (TCK30, Board of Radiation and Isotope Technology, Mumbai), Rest of the chemicals used in the present study were obtained from local Indian vendors. All chemicals were of analytical grade.

Study design

This study was approved by the Institutional ethics committee. A total of eight samples each were prepared as per vial and syringe method. The preparation of samples was repeated 5 times resulting in a total sample size of 80. For the image quality assessment, a study with cross-section design was conducted. A total of 100 patients were enrolled for the study and randomly divided into five groups, namely, Group I, II, III, IV, and V.

Group I served as control and patients were injected with Tc-99m MDP prepared as per conventional method (130 mCi (4.81GBq) Tc-99m (3 mL) added to the MDP kit and 18 mCi (666 MBq) Tc-99m MDP injected to the patient). Group II and III patients were injected with Tc-99m MDP, fractionated as per syringe method immediately on the same day (0 h) and on the next day (24 h) of reconstitution, respectively. Group IV and V; patients were injected with Tc-99m MDP, fractionated as per vial method immediately on the same day (0 h) and on the next day (24 h) of reconstitution, respectively.

Radiopharmaceutical preparation

The present study was carried out employing two different fractionation methods (vial method and syringe method), which are in common practice.

Vial method

In this method, 1.6 mL normal saline was added into a vial containing MDP (mother vial) the entire content of the reconstituted MDP vial was then withdrawn and 0.2 mL aliquots were dispensed into sterile vials. The vials were serially numbered as 0, 1, 2, 3,.....7.^[7] The 0 vial was used immediately after preparation and the remaining vials were stored at -20°C for future use.^[2] On the selected day, one of the frozen fractionated vial was removed from -20°C freezer and thawed at room temperature for 15–20 min.

For radiolabeling, Tc-99m pertechnetate was obtained as a fresh eluate from a 300 mCi (11.1 GBq) wet column generator. The required amount of radioactivity (18 mCi [666 MBq] approx.) (0.3 mL to 0.5 mL) was added to the selected fractionated vial.

Syringe method

In this method, 1.6 mL normal saline was added to the pharmaceutical MDP vial (mother vial). Every time 0.2 mL fractionate was transferred from the mother vial into a sterile syringe for radiolabeling and the remaining mother vial was stored at -20°C . The thawing and radiolabeling procedures were similar to as described above.

Quality control and image assessment

Each of these preparations (inclusive of vial and syringe method) were subjected to quality control checks which included physical appearance, pH, percentage radiochemical purity (%RCP) pyrogenicity, and sterility testing.

The %RCP of Tc-99m MDP was assessed by two solvent systems (acetone and saline) paper chromatography using commonly available paper chromatography module.^[4,8] A drop of Tc-99m MDP was spotted on one end (PS) of the Whatman paper strips (No 3) and placed inside the chromatography jar containing one of the solvents. The solvent was allowed to move upward through capillary action to 12 cm above PS. The strip was then taken out dried and cut into 1 cm paper segments. Each segment was read in gamma ray spectrometer attached to a well counter (GM611M, Nucleonix). The percent radiochemical purity was determined from a profile of the radioactivity distribution along the length of the strip.^[8]

Pyrogenicity testing was performed by standard qualitative gel clot LAL test as per the manufacturer's instructions. Sterility testing was performed by incubating radiopharmaceutical sample (100 μL) in a sterile thioglycollate broth at 37°C for 14 days. The media bottle was sterilized by autoclaving at 15 psi pressure and 121°C for 15 min then the media bottles were cooled to 25°C . Liquid thioglycollate medium (1 mL) was taken from the prepared media bottle and added into 5 mL culture media bottle (aerobic) with the addition of radiopharmaceutical.^[9] On the 14th day, the sample was observed (by preparing

smear) under a microscope. The same protocol was adopted for all samples.

Acceptance criteria for both vials and syringe methods are given in Table 1.

Image quality

The image quality and biodistribution produced by all methods were evaluated using quantitative and qualitative measures. The Tc-99m MDP in-vivo study was conducted on the samples to the 2nd day of the fractionation considering the deterioration of % RCP and statistical comparison of two groups.

Quantitative assessment

The quality of bone scan in all groups was assessed in terms of contrast, contrast-to-noise (CNR) ratio, and signal-to-noise ratio (SNR).^[10] All images were acquired in matrix. The region of interest (ROI) was drawn on the thigh bone and considered as target. The same dimension's ROI was also drawn on soft tissue for background measurements. The contrast of the image was measured as $C = (N_T - N_B)/N_B$, where N_T was the maximum number of counts measured in the ROI and N_B was the average background counts.^[10] CNR was calculated using $CNR = C / \text{COV}$ where COV, was the COV of noise. The SNR for the planar image was calculated as the square root of the average number of counts per pixel within the area occupied by the object.^[11,12]

Qualitative assessment

For qualitative assessment, the anterior and posterior images were interpreted by two independent physicians. Each reader assessed overall image quality on a 3-point scale (3, excellent; 2, good; 1, poor).^[13]

Statistical analysis

Radiochemical purity (%) was expressed as mean \pm standard deviation and readings greater or equal to 95% were considered acceptable. Image contrast, CNR, and signal-to-noise ratio were analyzed by one-way ANOVA followed by *post hoc* test (Dennett t 2 sided). The intra-reader agreement and inter-reader agreement for image quality assessment were analyzed by Crosstab, Kappa, and Chi-square test. $P \leq 0.05$ was considered statistically significant.

Table 1: Acceptance criteria for radiopharmaceuticals

Quality control checks	Normal
Physical appearance	Should not contain any particulate material
pH	6.5-7.5
Percentage RCP	\geq percentage RCP of Tc-99m MDP 95%
Pyrogenicity	Gel clot LAL assay, negative
Sterility test	No evidence of microbial growth [Figure 1]

RCP: Radiochemical purity, MDP: Methylene diphosphonate, LAL: Limulus Amebocyte Lysate

Results

Radiopharmaceutical preparation and quality control

Acceptance criteria for both vial and syringe methods are given in Table 1.

Vial method

The reconstituted Tc-99m MDP solution during all preparations was clear and colorless in appearance. The pH ranged from 6.5 to 7.5. The qualitative testing of endotoxins in the final labeled radiopharmaceuticals revealed the absence of endotoxins in the sample and the sterility test showed no evidence of microbial growth in any of the samples Figure 1. The vial fractionated Tc-99m MDP showed more than 95% RCP to the 4th day of fractionation and deteriorated to 83.6% by the 8th day of fractionation Figure 2.

Syringe method

The reconstituted Tc-99m MDP solution during all preparations was observed to be clear and colorless in appearance. The pH ranged from 6.5 to 7.5. The qualitative testing of endotoxins in the final labeled radiopharmaceuticals revealed the absence of endotoxins in the sample and the sterility test showed no evidence of microbial growth in any of the samples Figure 1. The vial fractionated MDP showed more than 95% RCP to the 2nd day of fractionation. The RCP (%) deteriorated to 88% by the 8th day of fractionation Figure 2.

Image quality

Quantitative assessment

The image contrast, CNR, and SNR were statistically similar for both syringe and vial fractionated MDP until the 2nd day postfractionation [Table 2]. These parameters remained unaffected to 4 days in the case of vial fractionated MDP (data not shown).

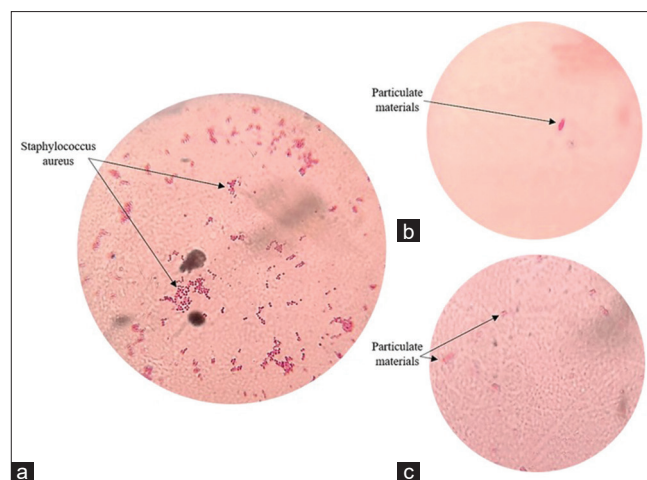


Figure 1: Microbial growth after 14 days: (a) Positive control (*Staphylococcus aureus*). (b) Vial method sample and (c) syringe method

Qualitative assessment

A good inter-observer agreement was noted among the observers. The images were analyzed on a 3-point rating scale. The data showed no statistical difference among images of vial and syringe fractionated MDP Table 3 and Figure 3.

Discussion

Tc-99m MDP is an extensively used radiopharmaceutical for skeletal imaging due to its higher bone accumulation.^[1] MDP is available in the form of ready to use radiolabeling kit, where radiolabeling is carried out by simply adding ^{99m}TcO⁴⁺ to the kit. The labeling yield is usually greater than 95% with a shelf life of 6–8 h postradiolabeling.^[5] Several vendors manufacture MDP kits and the composition of each kit varies from vendor to vendor in terms of quantities of the chelating ligand and the stannous ions present in the kit. While preparing ^{99m}Tc-labeled MDP, Tc-99m is added to the entire contents of a vial of reagent kit. The labeled compound is then withdrawn and subsequently, required dose is administered to the patients. This radiopharmaceutical preparation can be wasteful because of the short shelf life of the product formed after radiolabeling.^[2] The concept of unit-dosed nonradioactive reagent kits provides an efficient and cost-saving method for Tc-99m radiopharmaceuticals. MDP cold kits supplied by the companies are multi-doses vials and, after labeling with Tc-99m have a short shelf life. Therefore, if the sufficient number of patients does not report, then the majority of radiopharmaceutical preparation may go wasted.^[5] In such situations, unit dose preparations may be the most cost-effective approach. However, preparation

of unit doses requires assessment of proper stabilization, storage, radiochemical stability, and biological behavior of the radiolabeled product.

In the present study, we have assessed the effect of cold kit fractionation on the quality parameters of radiolabeled Tc-99m MDP. The kit contained 10 mg MDP, 5 mg NaCl, 0.5 mg gentisic acid, and 0.6 mg stannous chloride (BRIT kit). The quality tests conducted for the present study were physicochemical tests and biological tests. Physicochemical tests included the assessment of physical appearance of the final product, pH of the solution, radionuclide, and radiochemical purity. The biological tests included sterility and pyrogenicity testing. These tests are considered the gold standard for checking the quality of radiopharmaceuticals.^[4,14] All quality parameters were within the accepted range during all 8 days except RCP (%) parameter, which showed deterioration after 4 and 2 days of fractionation in the vial and syringe method, respectively. RCP (%) of the radiopharmaceutical depends on the stoichiometry, storage temperature of the kit, and the oxidative state of radiopharmaceuticals before and after the labeling.^[15-18]

In the syringe method, repeated thawing of the mother vial is needed and the integrity of vial may also get compromised by frequent needle piercing. These factors

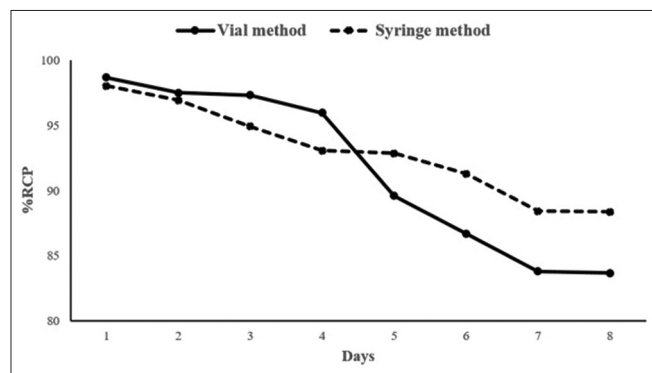


Figure 2: Percentage radiochemical purity variation with days, postfractionation in vial and syringe method

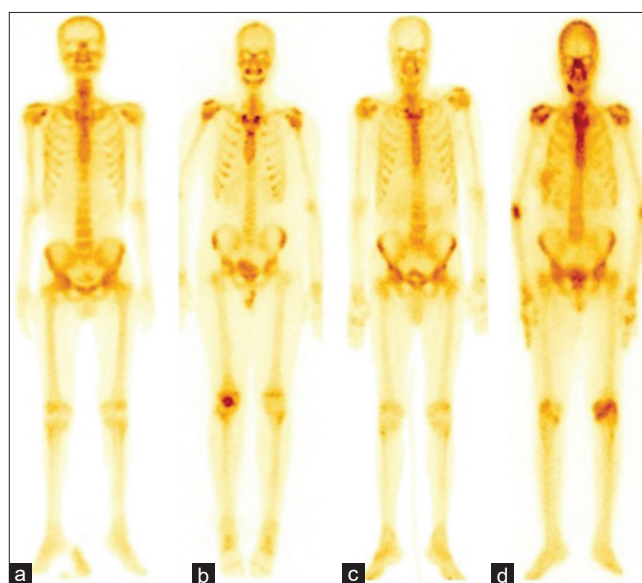


Figure 3: Whole body anterior bone image. (a) Day 1 (vial method). (b) Day 2 (vial method). (c) Day 1 (syringe method). (d) Day 2 (Syringe method)

Table 2: Quantitative image analysis: comparison with nonfractionated reconstructions of Tc-99m methyl diphosphonate images

	Nonfractionated	Vial fractionated		Syringe fractionated	
	Day 0	Day 1	Day 2	Day 1	Day 2
Contrast	1.34±0.32	1.34±0.33	1.35±0.37	1.39±0.24	1.37±0.44
Contrast-to-noise ratio	1.51±0.37	1.51±0.41	1.49±0.42	1.52±0.40	1.47±0.45
Signal-to-noise ratio	2.02±0.38	2.06±0.38	2.01±0.39	2.07±0.38	2.05±0.34

Table 3: Subjective image analysis

	Group				Total	
	Nonfractionated	Vial		Syringe		
		fractionated		fractionated		
		Day 1	Day 2	Day 1		Day 2
Reader 1						
Good	12	13	13	12	14	64
Excellent	8	7	7	8	6	36
Reader 2						
Good	12	12	13	14	14	65
Excellent	8	8	7	6	6	35
Total	20	20	20	20	20	100

might have contributed toward lower RCP (%) values observed in this method.^[13,16] However, these challenges are minimized in the case of the vial method.

The in-vivo study of fractionated Tc-99m MDP showed good quality bone scintigraphic images in both methods for tested durations. The results of the present study corroborated with the study conducted by Dhingra *et al.* 2019, wherein they have studied the effect of cold kit fractionation on image quality of MDP, DTPA, and DMSA kits and found that the biodistribution of all fractionated RPs showed normal behavior for up to 3 days.^[13] We had further made quantitative image analysis for the initial 2 days of postfractionation and observed no significant difference among both fractionation methods.

Conclusions

The present study was an attempt to understand the effect of different methods employed for cold kit fractionation on the quality of images produced by ^{99m}Tc-MDP. The observations revealed that if fractionated with utmost care both methods produced almost similar results. Although the present study reported the feasibility of cold kit fractionation. However, for each fractionation quality tests must be conducted before the administration of the radiopharmaceutical.

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Conflicts of interest

There are no conflicts of interest.

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