

Radiopharmaceutical, Original Article**Different Models for Cost-Effective Fractionation of Bone Radiopharmaceuticals; Methylene Diphosphonate (MDP) & Hydroxymethylene Diphosphonate (HDP)****Bayomy, T. * Abdulrazzak, M. ** Moustafa, H. *** Khalil, W.M. **** and Pant, G.S*******

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Abstract

The aim of this work is to study the availability of MDP & HDP cold kit fractionation with different modalities of different storage conditions and temperatures as a function of time elapsed after fractionation.

Samples were taken by adding a definite amount of 0.9NaCl solution to the cold kit and the modes of fractionation used were: 1) *Non-Oxidation condition, freezing (-20 to -28 °C) fractionation*, 2) *Non-Oxidation Condition, refrigerated (0 to 4°C) fractionation* 3) *Oxidation Condition, freezing fractionation*.

These modes of fractionation were studied for a period of 3 months depending upon the need of fractionation time for each radiopharmaceutical and on the changes in the results obtained for the different fractionation procedure. Instant Thin Layer Chromatography (ITLC-SG) with

Acetone & 0.9NaCl as solvents was simply used to determine the percentage Radiochemical Purity (%RCP) of the target ligand (^{99m}Tc -MDP & ^{99m}Tc -HDP) which was considered acceptable if greater than 95%. The concentration of the 2 main impurities TcO_4 & TcO_2 should not exceed 5%.

Results & Discussion:

MDP. The first mode of fractionation, all the samples studied with a mean %RCP of 98.37%, 98.56% and 98.69% for the first, second and third months respectively showed an excellent fractionation procedure under this condition. Refrigerator and syringe sampling modes of fractionation procedures were ultimately successful for three months under these conditions with a %RCP of 98.74% and 98.84% respectively.

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HDP: efficient fractionation procedure was verified in the first mode of fractionation with a %RCP of 98.28%, 98.08% and 98.07% for the first, second and third months of fractionation. In refrigerator fractionation mode, a mean %RCP of the HDP complex of 97.15% was obtained for 3 months study, showing a successful fractionation procedure for this long time period. Fractionation procedure for the syringe-stored samples showed fluctuating results with acceptable %RCP in the first and third months of 98.38% and 96.63% respectively and failed %RCP in the second month 88.88% respectively. Stannous augmentation was a good solution to overcome this problem as the degraded radiochemical purity was always accompanied with increase in the ^{99m}Tc -pertechnetate concentration.

Conclusion: MDP and HDP cold kits reflected an excellent behavior when fractionated for a period from 3 months with all studies fractionation modules except the fluctuated results of HDP with the oxidation condition fractionation, which can be recovered by augmentation of stannous ions. The practicality of the second fractionation mode should be studied from the sterility point of view.

Keywords: MDP&HDP, Fractionation, Radiopharmaceuticals

Introduction

In developing countries, it is generally planned to perform the scanning of any specific organ or similar studies in one day, which is dependant on the number of patients and the availability of ^{99m}Tc activity (*Mansur, 2006*). In small nuclear medicine set up, the number of patients is limited. In big departments also single patient may be accepted for the performance of a study of unscheduled nature. In both these situations the availability of single patient doses

becomes necessity to avoid the employment of the cold kit for a single patient.

MDP and HDP cold kits are relatively not expensive; however, the missuse of these kits in the form of implementing the whole vial which can accommodate activity enough for 25 patients to be used for one or two patients raised the idea of their fractionation.

In addition, simple methods of fractionation need to be implemented to avoid the use of chemical stabilizers (*Hung, 2002*), (*Siddig, 2002*) and (*Solanki, 1994*) or antioxidants (*Mansur, 2006*) in the patient doses or employment of sophisticated storage conditions like very low temperatures (-70°C) (*Morrissey, 1993*) or using isolating agents like argon gas or liquid nitrogen.

Materials & Methods

The aim of this study was to determine the best conditions and arrangements that need to be followed to obtain a good cold kit fractionation procedure for the 2 mentioned ligands.

Fractionated samples were stored in 3 different conditions. *In the first one*, the fractionated portions were transferred to sterile evacuated vials and kept in the freezer at a temperature between -20 to -28°C . *The second method* includes storing of the evacuated-vial fractions in a refrigerator at a temperature of $0-4^{\circ}\text{C}$. *In the third method*, fractionated portions were kept in syringes and stored in the freezer (-20 to -28°C). Accordingly we were able to test the efficiency of the oxygen protected frozen and refrigerated fractions as well as the oxygen unprotected fractions (oxidation condition) respectively as a function of the time elapsed after fractionation.

Other experiments were done to find a) the appropriate methods of radiochemical purity testing for each radiopharmaceutical, b) to test the efficiency of the different methods of measurement of radioactivity in thin layer

chromatography (TLC), c) to assess the effect of cold kit batch variation on the efficiency of the fractionated cold kits.

All studied cold kits were subjected to the following steps:

1-The sampling procedure was achieved by adding with the smallest available needle (27G) a known amount of non-bacteriostatic saline (low dissolved-oxygen (LDO) saline was not used because it is normally not available in all nuclear medicine facilities) to the swapped vial of the desired kit. A **Reference Fraction** was always labeled immediately for getting the standard radiochemical purity values for the fractionated kit to assure its suitability for fractionation and to be compared later with the other fractionated samples.

Within one minute, the fractions stored in syringes would go to the freezer along with the evacuated vial protected-fractions that are subjected to freezing (-20°C to -28°C), while the others used for assessing the ability of storing the fractions in higher temperature levels were put in the refrigerator (0°C to 4°C).

2-Labeling procedure is the reconstitution of the fractionated samples with sterile, non-pyrogenic, oxidant free and quality control passed sodium pertechnetate ^{99m}Tc solution.

Chromatography: After a proper incubation time suggested by the manufacturer in the package insert, a specific chromatography procedure was applied immediately to determine the radiochemical purity of the studied fraction. The chromatography module chosen in this research was the Thin Layer Chromatography (TLC) since it is applicable in most nuclear medicine facilities because

of the availability of its components and its relatively accurate results.

The percentage radiochemical purity was determined when a profile of the radioactivity distribution along the length of the strip was created with the gamma camera software where the areas under the peaks of the different species were measured. Another simple method was used by determination the total count for each species by drawing of the region of interest (ROI) containing that activity

^{99m}Tc - **Methyiene Diphosphonate (MDP.)**

The commercial kit (*Medronate II, GE Healthcare, UK*) was reconstituted with 5.0 ml of 0.9NaCl then fractionated to 10 equal fractions each of 0.5ml.

50mCi of $^{99m}\text{TcO}_4^-$ in 0.5 ml of age less than 6 hours was used for the fractionated sample, since the maximum activity instructed by the manufacturer is 500 mCi (18.5GBq). The labeling was done according to the instructions given in the package insert.

After 15 minutes of incubation time for ^{99m}Tc -MDP, a 2-system chromatography procedure (Figure 2) was applied (**Saha, 2004**): System I: ITLC-SG / Acetone And System II: ITLC-SG / Saline (0.9NaCl) Where, % Pertechnetate ($^{99m}\text{TcO}_4^-$) = % activity at the solvent front in System I. % Reduced-hydrolyzed Tc ($^{99m}\text{TcO}_2^-$) = % activity remaining at the origin in System II % ^{99m}Tc -MDP = (% activity at the solvent front in System II) - % Pertechnetate ($^{99m}\text{TcO}_4^-$) or, = (% activity remaining at the origin in System I) - % Reduced-hydrolyzed Tc ($^{99m}\text{TcO}_2^-$).

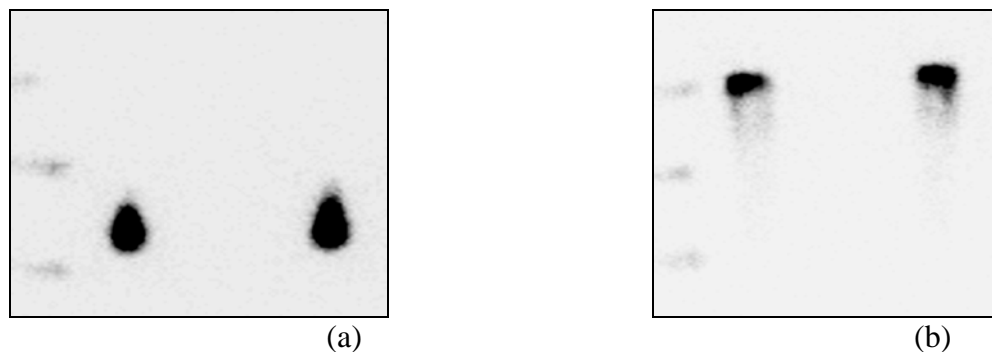


Figure (1): Radioactivity distribution along the strips of the two chromatography systems used for radiochemical purity determination of ^{99m}Tc -MDP where a) System I: ITLC-SG / Acetone, b) System II: ITLC-SG / Saline. The left-sided three hot spots in the two figure parts showing point source markers at $R_f = 0$, $R_f = 0.5$ and $R_f = 1$.

^{99m}Tc -Hydroxymethylene Diphosphonate (HDP)

The commercial kit (*TechneScan*HDP, Mallinkrodt Medical B.V*) was fractionated to 8 equal fractions each of 0.5 ml by adding 4ml of sterile saline solution.

25mCi of $^{99m}\text{TcO}_4^-$ in 0.4 ml with age less than 6 hours was used for the 8 fractionated samples, since the maximum activity instructed by the manufacturer is 200 mCi (7.4GBq). The labeling was done according to the instructions given in the package insert.

After the incubation time for ^{99m}Tc -HDP, the procedure applied for ^{99m}Tc -MDP radiochemical purity testing was also done here (**Saha, 2004**).

N.B. The activity in the solvent front appeared in all experiments at $R_f = 0.5$ -1.0 (Figure 7.9) which is not mentioned by **Saha (2004)**, were compared with the old/conventional standard method (**Krogs-gaard, 1976** and **Zole, 2007**) verifying that, the mentioned area in this strips should be considered as solvent front.

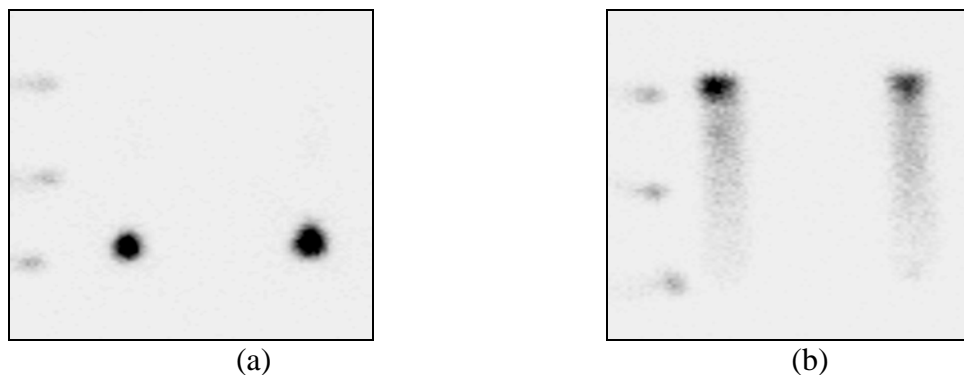


Figure (2): Radioactivity distribution along the strips of the two chromatography systems used for radiochemical purity determination of ^{99m}Tc -HDP where a) System I: ITLC-SG / Acetone, b) System II: ITLC-SG / Saline. The left-sided three hot spots in the two figure parts showing point source markers at $R_f = 0$, $R_f = 0.5$ and $R_f = 1$.

Results & Discussion

^{99m}Tc-MDP

^{99m}Tc-MDP is extensively used in most nuclear medicine departments since its introduction by *Subramanian et al* in 1975. ^{99m}Tc-MDP has been commonly used for a long period as the pharmaceutical of choice for bone scan imaging (*McAfee, 1987*); recently its role in detection of breast masses has also been proven (*Chen, 2007*).

Commercial kit formulations contain various quantities of active ingredients (*Tofe et al, 1980*). Reduced, hydrolyzed ^{99m}Tc has been a common impurity which can be caused by aluminum in ^{99m}Tc- elute, so optimal labeling conditions can be obtained from a ^{99m}Tc- elute of age less than six hours from a generator that is eluted daily (*Ponto et al, 1987*).

In the present study, ^{99m}Tc-MDP preparations labeled from frozen, oxidation protected fractionated samples up to 3 months as well as refrigerator and syringe stored samples which studied and compared to the reference values where a percentage radiochemical purity % RCP greater than 95% is only accepted for a successful preparation (*European Pharmacopoeia, 2005*), (*US pharmacopeial convention, 2005*) and (*G. International pharmacopeia, 2007*).

Non-Oxidation condition, freezing fractionation

Reference Samples Mean and standard deviation (SD) values (n=40) for the % RCP of ^{99m}Tc-MDP complex (98.94 +/- 1.02), free ^{99m}Tc (0.27 +/- 0.31) and hydrolyzed reduced ^{99m}Tc (0.79 +/- 0.89) showed excellent radiochemical purity validating the acceptance of these preparations as reference

for the fractionated samples. The determined % RCP values for the reference samples agree with those of *McAfee (1987)* and *Ponto et al. (1987)*.

First Month Samples Mean and standard deviation (SD) values (n=54) for the % RCP of ^{99m}Tc-MDP complex (98.37 +/- 1.83), free ^{99m}Tc (0.42 +/- 0.94) and hydrolyzed reduced ^{99m}Tc (1.21 +/- 1.23) showed typically matched values with those of the reference samples with the insignificant p-value of 0.077 and 0.337 and 0.07 respectively.

Second Month Samples Mean and standard deviation (SD) values (n=48) for the % RCP of ^{99m}Tc-MDP complex (98.56 +/- 1.27), free ^{99m}Tc (0.34 +/- 0.45) and hydrolyzed reduced ^{99m}Tc (1.09 +/- 1.19) showed identical values with those of the reference samples assuring the efficiency of the MDP cold kit fractionation under these conditions for the second month. This was reflected by the insignificant p-value of 0.129, 0.408 and 0.192 respectively.

Third Month Samples Mean and standard deviation (SD) values (n=48) for the % RCP of ^{99m}Tc-MDP complex (98.69 +/- 1.16), free ^{99m}Tc (0.47 +/- 0.54) and hydrolyzed reduced ^{99m}Tc (0.84 +/- 1.04) and p-values of 0.296, 0.043 and 0.813 respectively, showed the identical values as those of the reference preparations with a very minimal change in the ^{99m}Tc-pertechnetate concentration which can be safely neglected. Table (1) summarize the %RCP of the frozen, oxidation protected ^{99m}Tc-MDP labeled during the 3 months period of the study.

Table (1): Mean values for %RCP of frozen, oxidation protected ^{99m}Tc -MDP fractionated samples labeled in the 3 months period of the study.

	Reference	1 st Month	2 nd Month	3 rd Month
Labeled MDP	98.94	98.37	98.56	98.69
Free Tc	0.27	0.42	0.34	0.47
HR Tc	0.79	1.21	1.09	0.84

Non-Oxidation condition, Refrigerated fractionation (3 months)

Mean and standard deviation (SD) values ($n = 30$) for the % RCP of ^{99m}Tc -MDP complex (98.74 +/- 0.79), free ^{99m}Tc (0.28 +/- 0.34) and hydrolyzed reduced ^{99m}Tc (0.98 +/- 0.73) reflected typical %RCP values with those of the reference samples verified by insignificant p-values for the three studied species of 0.375, 0.899 and 0.344 respectively.

Oxidation condition, freezing fractionation (3 months)

Mean and standard deviation (SD) values ($n = 26$) for the % RCP of ^{99m}Tc -MDP complex (98.48 +/- 1.10), free ^{99m}Tc (0.50 +/- 0.57) and hydrolyzed reduced ^{99m}Tc (1.02 +/- 0.77) and p-values of 0.087 and 0.038 and 0.286 respectively, showed identical values as those of the reference preparations with a very minimal change in the ^{99m}Tc -pertechnetate concentration which can be safely neglected.

The previously presented results reflect the efficiency of MDP cold kit fractionation with the three studied modules for a period of three months. These efficient fractionation procedures obtained here can be attributed to the high tin concentration in addition to the presence of excessive antioxidants on the commercial cold kit allowing a sufficient concentration of tin for TcO_4 reduction process.

^{99m}Tc -HDP

^{99m}Tc -MDP or HMDP is the preferable agent for bone imaging in nuclear medicine. It showed favorable characteristics in early studies comparing with MDP (*Francis Et al, 1980 & Fogelman et al, 1981*). However after critical evaluation it was concluded that there was no significant difference between HDP and MDP (*Zolle, 2007*).

Presence of aluminum in ^{99m}Tc elute may result in formulation of colloidal products (TcO_2) which may interfere with the image by changing the distribution of the activity to be partially localizing in liver, spleen and lung (*Zimmer, 1978*).

All ^{99m}Tc -diphosphonate agents are weak chelates and tend to degrade with time, producing TcO_4 by the action of oxygen and radiation produced free radicals. This problem had been treated in all commercial cold kits by adding excess tin, and /or antioxidants (*Saha, 2004*).

The same chromatographic procedures applied for MDP with the same %RCP limits were implemented with HDP fractionation samples.

Non-Oxidation condition, freezing fractionation

Reference Samples Mean and standard deviation (SD) values (n = 48) for the % RCP of ^{99m}Tc -HDP complex (98.21 +/- 1.23), free Tc99m (0.30 +/- 0.32) and hydrolyzed reduced Tc99m (1.49 +/- 1.17) showed excellent radiochemical purity validating the acceptance of these preparations as reference for the fractionated samples. Similar to MDP, the determined % RCP values for the reference samples agree with those of *McAfee (1987)* and *Ponto et al. (1987)*.

First Month Samples Mean and standard deviation (SD) values (n = 44) for the % RCP of ^{99m}Tc -HDP complex (98.28 +/- 0.91), free Tc99m (0.29 +/- 0.46) and hydrolyzed reduced Tc99m (1.41 +/- 0.91) as well as the highly insignificant p-values of (0.759), (0.903) and (0.717) respectively, validate a highly efficient HDP fractionation procedure under this condition for one month storage time.

Second Month Samples Mean and standard deviation (SD) values (n = 34) for the % RCP of ^{99m}Tc -HDP complex (98.08 +/- 1.45), free Tc99m (0.37 +/- 0.62) and hydrolyzed reduced Tc99m (1.56 +/- 1.40) and p-values of 0.663, 0.506 and 0.806 respectively, with identical values to those of the reference preparations.

Third Month Samples Mean and standard deviation (SD) values (n = 40) for the % RCP of ^{99m}Tc -HDP complex (98.07 +/- 1.34), free ^{99m}Tc (0.16 +/- 0.22) and hydrolyzed reduced ^{99m}Tc (1.76 +/- 1.26) and p-values of 0.611, 0.021 and 0.313 respectively, showed identical values to those of the reference preparations with an ultimately ignorable decrease in the ^{99m}Tc -pertechnetate concentration. Table (2) summarize the %RCP of the frozen, oxidation protected ^{99m}Tc -MDP samples labeled during the 3 months period of the study.

Table (2): mean values for %RCP of frozen, oxidation protected ^{99m}Tc -HDP fractionated samples labeled in the 3 months period of the study.

	Reference	1 st Month	2 nd Month	3 rd Month
Labeled HDP	98.21	98.28	98.08	98.07
Free Tc	0.30	0.29	0.37	0.16
HR Tc	1.49	1.41	1.56	1.76

These results confirm the successful fractionation of HDP cold kit under this low temperature (-20 to -28°C), oxidation protected condition for a period up to three months.

The stability of the fractionated samples up to this time can be definitely attributed to the same arguments given in MDP fractionation which is the presence of excessive tin concentration; efficient antioxidants and suitable generator elute (*Ponto et al, 1987*).

Non-Oxidation condition, Refrigerated fractionation (3 months)

Mean and standard deviation (SD) values (n = 26) for the % RCP of ^{99m}Tc -HDP complex (97.15 +/- 1.91), free ^{99m}Tc (1.07 +/- 1.48) and hydrolyzed reduced ^{99m}Tc (1.78 +/- 1.48) and p-values of (0.005), (0.001) and (0.329) respectively, showed a minimal decrease in the %RCP of the ^{99m}Tc -HDP accompanied with an equal increase in the ^{99m}Tc -pertechnetate concentration while TcO_2 concentration showed no changes compared with the reference value.

These results represent the safe fractionation of the HDP kit under this condition up to 3 months.

Oxidation condition, freezing fractionation (3 months)

The unsystematic data obtained from these results prevent the application of syringe storage fractionation beyond the first month even with stannous augmentation since we got one case of radiochemical purity failure in the third month due to extensive concentration of the hydrolyzed, reduced ^{99m}Tc .

No particular arguments can be but to explain this changed behavior of HDP syringe stored fractions against the complete fractionation success obtained with MDP under similar conditions.

Finally, the following notes are very critical with any cold kit fractionation practice:

1. Sterility is an essential issue when applying the refrigerator fractionation. All fractionation procedures can only be done under aseptic conditions, however the temperature at 0 to 4°C is not a suitable condition for maintaining 100% sterility for the stored solution.
2. Stannous augmentation can be very helpful in many fractionation failure conditions, however, stannous concentration is a critical issue in the drug safety and labeling efficiency in many radiopharmaceutical. So, an extremely precise estimation of augmented stannous has to be achieved.
3. It is very important to mention that, the fractionation procedure should be carried out by qualified and skilled personnel with good understanding of the different radiopharmaceutical labeling parameters and accurate quality control program.

Conclusion

MDP and HDP cold kits reflected an excellent behavior when fractionated for a period from 3 months with all studies fractionation modules except the fluctuated results of HDP with the oxidation condition fractionation which can be recovered by augmentation of stannous ions.

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PHYSICS, Original Article**Investigation of Multiple Head Registration / Center of Rotation for SPECT Gamma Cameras**

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Abstract

Purpose: One of the important quality control checks for SPECT Gamma Camera is the centre of rotation (COR). Multiple Head Registration / Center of Rotation (MHR/COR) is also important not only to observe the mechanical errors in the state-of-art Gamma Camera, but also to correct, quantitatively, the errors from the movement of patients and detectors on the SPECT images.

Material and Method: Three gamma cameras (two Siemens and one Philips), were studied using Low Energy High Resolution (LEHR) collimators with protocols provided by the manufacturers based on the international regulations and committees.

In Siemens cameras (Ecam and Duet), MHR/COR was studied using five point sources of Tc99m (1 mCi) each in a special phantom. In Philips camera (Forte), COR was measured using an assembly consisting three point sources of Tc99m (0.5 – 1 mCi) with the Jet stream Quality Assurance (QA) software.

Results: The MHR/COR was studied in Siemens cameras (Ecam and Duet) including the error of X- max., X-min., Y shift and back projection angle with 180 and 90 degrees configurations for 30 months. Ecam results showed high stability through this period but Duet values are slightly varied. The results of COR in Forte camera including the error of X- max., X-min and Y error range with 180 and 90 degree configurations indicated marked changes within 26 months. However these changes were observed within the acceptable limits.

Conclusion: The MHR/COR quality control checks are crucial indication about the mechanical performance of a SPECT camera. It is important to update the correction map of the software to correct the camera heads registration errors and the patients movements. It is recommended for Forte to measure the COR on weekly basis in order to maximize the benefit from the COR calibration correction software.

Key words: SPECT Gamma Camera; MHR/COR; Quality Control

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