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PREPARATION AND QUALITY CONTROL OF ^{99m}Tc-MDP

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ABSTRACT

Since the early 1970s a number of ^{99m}Tc-phosphate compounds have been introduced for bone imaging. The three extensively studies diphosphonates are: 1-hydroxiethylidene diphosphonate (HEDP), methylene diphosphonate (MDP) and hydroxymethylene diphosphonate (HMDP or HDP) of which MDP and HDP are most commonly used in nuclear medicine. This report describes the routine production and quality control of MDP ("multidose") at IPEN-CNEN/SP, in lyophilized form for labeled with high activities of ^{99m}Tc (9,250 MBq).
The process was done under vacuum and low temperature in Super Modulyo –"Edwards" lyophilizator and each lyophilized vial contains: 5.0 mg of MDP; 1.0 mg $SnCl_2.H_2O$; 0.1mg ascorbic acid and 20.0 mg sodium pyrophosphate at pH = 6.0.
The radiochemical purity was determined by thin layer chromatography system in Whatman 3MM paper (1 x 8 cm), using acetone and saline (0.9% NaCl) as solvents, respectively. Sterility and pyrogen tests were performed by the microbiology procedures outlined in the pharmacopoeias and by the "in-vitro" Limulus test, respectively. Biological distribution in Wistar rats was evaluated in different organs (% dose / organ).
The method was validated for routine production at Radiopharmacy Center, the radiochemical purities of ^{99m}Tc-MDP ("multidose") was > 95%. The biological distribution in rats showed high uptake in bone tissue, and low activities in thyroid, liver and stomach. Sterility and pyrogen tests were negative in all the delivered lyophilized vials.

1. INTRODUCTION

Nuclear applications in health care can be roughly divided into diagnostic, therapeutic, and preventive applications. Diagnostic applications are a major nuclear medicine technology. The principal role of these procedures is the assessment of organ function. Radionuclides or compounds that are labeled with radionuclides are administered to the patients to allow specific organ function to be evaluated by tracing the dynamic biodistribution of such a compound in specific organs. Tracing of the compound is achieved by external detection of the photon emitted from the radionuclides by using instruments like rectilinear scanners or gamma cameras, SPECT (Single Photon Emission Tomography) and PET (Positron Emission Tomography.
Probably the major characteristic of in vivo nuclear medicine procedures is that the amount of radiopharmaceutical needed for in vivo diagnostic studies is very tiny and always in the range of physiological quantities.
Bone scanning is probably the technique most commonly performed. This is a very efficient approach to look at the function of the bone: any significant injury to the bone will cause an

increase in bone metabolism that can be detected by the bone scan. The method is the most efficient way to search for bone metastases in many types of tumors. It is also the study of fractures, osteomielites, Paget's disease or joint replacements [1]. Phosphonate ligands formed the basis for therapy in such bone disorders as Paget's disease, and this targeting ability brought such ligands to the attention of Subramanian and others in the early 1970s. The diphosphonates were amenable to the recently discovered instant kit formulation, and several agents became commercially available [2-3]. The first application of technetium as a radiopharmaceutical was using the $^{99m}Tc0_4^-$ eluted from a $^{99}Mo/^{99m}Tc$ generator to obtain images of the thyroid, liver and brain. Subsequently an "instant kit" was developed by Ekelman, Atkins and Richards, in which a single vial containing both a reducing agent (stannous chloride) and a complexing agent produced ^{99m}Tc-complexes in high radiochemical yield in sterile aqueous solution. [4-5-6]. This led to the development of early technetium agents such ^{99m}Tc-MDP used for skeletal imaging by several authors.
The objective this report is describes the routine production and quality control of MDP lyophilized form for labeled with high activities of ^{99m}Tc (9.250 MBq).

2. MATERIALS AND METHODS

2.1. Preparation of MDP lyophilized form

A lyophilized formulation is prepared from a "master solution" of MDP (Plenum) in an acidic solution of $SnCl_2.2H_20$ (Merck) in appropriate proportion (5:1). The pH of the solution is adjusted to 6.0 with 1N NaOH, purged with nitrogen, sterilized by Millipore filters (0.22μm) and aliquots of 1mL dispensed into individual kits vials. The product (1ml) was lyophilized under vacuum and low temperature in the "Supermodulyo Lyophilizator Edwards" during 24 hs. Each vial contains: 5.0 mg MDP (methylene diphosphonate acid), 1.0 mg $SnCl_2.2H_2O$, 0.1 mg ascorbic acid and 20 mg pyrophosphate, at pH 6.0. The preparation is carried out using sterile materials and under strict aseptic conditions in a laminar flow hood filled under positive pressure.

2.2. Labeling and Radiochemical Purity Determination

The lyophilized product was labeled with sodium pertechnetate elutes from ^{99}Mo – ^{99m}Tc generator (IPEN) with 7,400 – 9,250 MBq of ^{99m}Tc (3-5ml). The vial was shaken until completely dissolution), kept at room temperature and the radiochemical purity was carried out by paper chromatography system 30, 60, 120 and 240 minutes after labeling. A combination of two chromatography solvents were used: acetone and sodium chloride 0.9% solution in Whatman 3MM paper strips of 1,0 x 8,0 cm size. The R_f values in acetone is 1.0 for $^{99m}Tc0_4^-$ and 0.0 for $^{99m}Tc0_2^-$ / ^{99m}Tc-MDP and in 0.9% NaCl is 1.0 for $^{99m}Tc0_4^-$ / ^{99m}Tc-MDP and 0.0 for $^{99m}Tc0_2$. Then dry the chromatograms and after drying counted each segments in a gamma counter "Packard-Cobra II". The results are expressed as a percentage of the total count of the strip. The stability was evaluated during 6 months and the validation performed in 5 batches.

2.3. Biodistribution Study

^{99m}Tc-MDP was administered intravenously (240 MBq/0,1mL) in Wistar rats (250 –300g). The animals were sacrificed at 2 hours and tissues of interest removed: femur, liver, kidney and intestine, washed and weighted. Radioactivity was determined in a gamma counter (Packard). The results were expressed as percent administered dose/organ. The femur activities represent 10 % of the total activities of skeleton.
The "in-vivo" localization of ^{99m}Tc-MDP was observed in a mini-gamma camera "Berthold-Gamma Budapest, MB9420 after 2 hours of intravenously dose of 55.5 MBq / 0.2mL in Wistar rats.

2.4. Microbiological Study

The microbiological analysis was determined in different culture medium (sodium tioglicolate and Soybean casein tripticase broths) incubated at room temperature and at (35 ± 2)º C. The apirogenicity is evaluated using the "in-vitro" Limulus test (LAL).

3. RESULTS

3.1. Radiochemical Purity Determination

The radiochemical purity of ^{99m}Tc-MDP (Table 1) at 30, 60, 120 and 240 minutes after labeling were (98.54 ± 0.7)%; (98.51 ±1.0)%; (98.26 ± 0.6)% and (97.79 ± 0.9)%, respectively, using 7.400 – 9.250 MBq of ^{99m}Tc in 3 -5ml. The stability was evaluated during 6 months (Table 2).

Table 1 - Radiochemical Purity of ^{99m}Tc-MDP

Time (minutes)	% ^{99m}Tc-MDP	% TcO_2	% TcO_4^-
30	98.54 ± 0.7	1.40	0.06
60	98.51 ±1.0	1.42	0.07
120	98.26 ± 0.6	1.66	0.08
240	97.79 ± 0.9	2.16	0.05

(n = 3)

Table 2 - Radiochemical stability of ^{99m}Tc-MDP

Time (months)	% ^{99m}Tc-MDP	% TcO_2	% TcO_4^-
1	98.11 ± 0.2	1.85	0.04
3	98.10 ± 0,5	1.86	0.04
6	98.17 ± 0.7	1.76	0.07

(n = 3; 30 minutes)

In the validation studies (5 batches), free [^{99m}Tc] was less than 1% and [$^{99m}Tc0_2$] less than 3 %. The radiochemical purity was higher than 95 % at 30 minutes after labeling, as illustrate Table 3 [1].

Table 3 - Validation study ^{99m}Tc-MDP

Batch	% ^{99m}Tc-MDP	% TcO_2	% TcO_4^-
1	97.86 ±0.7	2.09	0.05
2	98.54 ± 0.2	1.40	0.06
3	98.64 ± 0.5	1.29	0.07
4	98.15 ±0.2	1.77	0.08
5	97.88 ±0.4	2.07	0.05

(Time = 30 minutes; n = 3)

3.2. Biodistribution Study

High uptake in skeleton was observed (90.94±1.9)% in Table 4 with maximum activities (2.41 ± 0.7)% in kidney at 30 minutes and (2.64 ±0.7)% and (3.35± 1.1)% in intestine and liver, respectively (n = 3). The biological distribution studies have presented compatible values with the literature as shown Figure 1, a much smaller quantity of injected ^{99m}Tc-MDP binds to the blood plasma proteins, which results in a very slight body background.
^{99m}Tc-MDP not bound to the skeleton washes out (50 %) from the body in the urine. Washout through the hepatobiliar system is negligible [7].

Table 4. Biological distribution in different organ (% dose / organ).

Organ	% Activity
Skeleton	90.94 ± 1.9
Liver	3.35± 1.1
Kidney	2.41 ± 0.7
Intestine	2.64 ±0.7

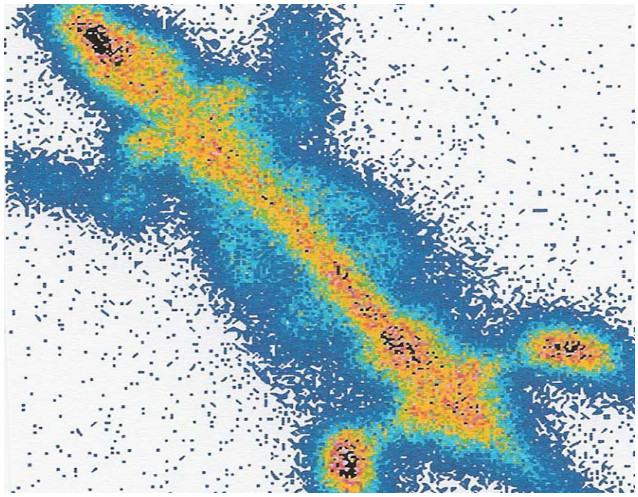

Figure 1 – Biological distribution of ^{99m}Tc-MDP

4. CONCLUSIONS

The lyophilized preparation, labeling and quality control procedures for ^{99m}Tc-MDP have developed, validated and simplified to extend it to large-scale productions at IPEN-CNEN/SP. In the radiochemical analysis low level of free ^{99m}Tc and $^{99m}TcO_2$ were detected and the pyrogen and microbiological tests were negative in all samples. The kit has proved satisfactory with a shelf life of 6 months and with a high yield of radiochemical purities after 4 hours of labelling. During 2004 were distributed 10,800 MDP "kit" (5 vial / kit) to Nuclear Medicine Hospitals and Clinics in Brazil.

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