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RADIOCHEMICAL PURITY OF ^{99m}Tc-MIBI X ACTIVITY QUANTIFICATION IN THE LIVER AND THE HEART

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ABSTRACT

Among the diagnostic procedures of nuclear medicine, the myocardial perfusion scintigraphy has stood out for the detection of arterial coronary disease. To perform this scintigraphy it is used ^{99m}Tc-MIBI radiopharmaceutical, where MIBI consists of a lyophilized kit for parenteral use and is labeled with ^{99m} Tc in Nuclear Medicine Services (NMS). According to the RDC nº 38 published by the National Health Surveillance Agency – ANVISA in 2008, a minimum of tests should be performed with the radiopharmaceutical before the administration in patients, to ensure their proper biodistribution and therefore good quality examination. Thus, this study aimed to evaluate the radiochemical purity (PRQ) of ^{99m}Tc-MIBI radiopharmaceutical in three NMS in the city of Recife/Brazil, relating it to the activity quantification in the target organs (heart and liver). The quality test was performed by thin layer chromatography, following the existing protocol in the instructions of the ^{99m}Tc-MIBI manufacturers. To evaluate the biodistribution, 5 minute-still-images from the chest/abdomen of 20 patients by SMN were acquired and the present activities in the heart and liver were quantified and then the relations A_{liver} / A_{heart} were calculated. The results showed that, in two evaluated SMN (B and C), samples of the ^{99m} Tc-MIBI radiopharmaceutical had a PRQ value outside the acceptance limits (≥90%). Regarding the measurement of the activity, a service (A) showed four images with the ratio ratio A_{liver} / A_{heart} higher than 1, while the other two showed two images each. Despite the service C have shown percentage of PRQ lower than 90% in 3 marked kits, it presented a proportion A_{liver} /A_{heart} lower than the average of the other services. This seems to show that the proportion A_{liver} /A_{heart} is not a factor that depends on the radiochemical purity.

1. . INTRODUCTION

Nuclear Medicine is a medical specialty that uses radioactive materials for diagnostic or therapeutic purposes. Obtaining the diagnosis of organ or tissue consists of administering intravenously, but it can also be by oral or by the inhalation of a chemical substance labeled with a gamma emitting radionuclide or positons which has affinity for the organ or the tissue that has been investigated [1]. Among the many procedures of Nuclear Medicine diagnostics, the myocardial perfusion scintigraphy has been highlighted in recent years and to carry out this procedure, one of the radiopharmaceuticals used is the 2-methoxy 2-isobutyl Isonitrile (MIBI) labeled with technetium (^{99m}Tc-MIBI).

As the drugs labeled with ^{99m}Tc consist of lyophilized kits for parenteral use, that are handled in Nuclear Medicine Services (NMS), it is necessary to carry out a quality test. According to the RDC nº 38 by National Surveillance Agency - ANVISA in 2008 [2], a minimum of tests should be performed with the radiopharmaceutical before the administration in patients, to

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ensure their proper biodistribution and therefore good quality examination [3].

Thus, this study aimed to evaluate the radiochemical purity (PRQ) of 99mTc-MIBI radiopharmaceutical in three NMS in the city of Recife/Brazil, relating it to the activity quantification in the target organs (heart and liver).

2. MATERIALS AND METHODS

This study was approved by the Ethics Committee of research involving human beings at the Federal University of Pernambuco under number CAAE 07040912.1.0000.5208 and report number 179.539. Three SMN were selected in Recife – PE-Brazil. In each one of them, the quality control in ^{99m}Tc-MIBI radiopharmaceutical of two different national manufacturers was performed.

The NMS, which were named as services A, B and C, released a different number of patients per day, an average of 5 patients in service A and 2-3 in services B and C. Consequently, services B and C radiopharmaceutical quality tests were conducted with a larger number of samples (6 and 8 samples, respectively) comparing to service A (4 samples).

Twenty patients in each service were studied for about three weeks after signing the "Termo de Consentimento Livre e Esclarecido" (Agreement Form). It is important to highlight that each NMS had different protocols for the use of myocardial scintigraphy, both regarding the administered activity and the phase order (stress/rest).

Biodistribution was evaluated by quantifying the activity present in the organs of interest (heart and liver), using the technique of combined images [4]. For the ^{99m}Tc-MIBI radiopharmaceutical handling, radiological protection equipment were used, as lead cloak, thyroid shield and gloves.

2.1. Evaluation of ^{99m}Tc-MIBI Radiochemical Purity

The assessment of the radiochemical purity was performed for the two national MIBI drug manufacturers, based on the methodology described in the package insert of the manufacturers. A total of three Whatman paper tape strips and three silica gel of 1 cm width and 6.5 cm length, labeled in a distance of 1 cm from the base to the sample application point of the ^{99m}Tc-MIBI radiopharmaceutical, as shown in Figure 1 (1A and 1B).

Then, the three Whatman strips were placed in glass bottles containing 1 ml of methanol solvent, and the three silica gel tapes were placed in vials containing 1 mL of the sodium chloride (NaCl) solvent (Figure 1C). After 10 minutes, the strips were removed, placed in a tray and evaluated by two different detection methods. In the first method, the scintillation chambers of the SMN participants were used, where the tray with ribbons was placed on the examination table and acquired a still image [4]. This was performed with a low energy collimator, in a distance of 10 cm between the tapes and the collimator in a 180-second-acquisition-time, as shown in Figure 1D.

Later, two areas were defined, better known as Region of Interest (ROI - Region of Interest), from the base of the tape to 2.5 cm and the other from 2.5 cm to the rest of the tape as shown

in Figure 1E and 2. Count rates were obtained in each selected region, then calculations to get the radiochemical purity PRQ were made for both methods by equation 1.

$$PRQ = 100 - (\%\ ^{99m}TcO^{-4} + \%\ ^{99}TcO_2) \quad (1)$$

where: $^{99m}TcO^{-4}$% is the percentage of free sodium pertechnetate present in the sample of the radiopharmaceutical, in the bottom of silica gel tape; % $^{99}TcO_2$ is the percentage of this reduced-hydrolyzed technetium radionuclide in the sample, in the top of the Whatman ribbon paper.

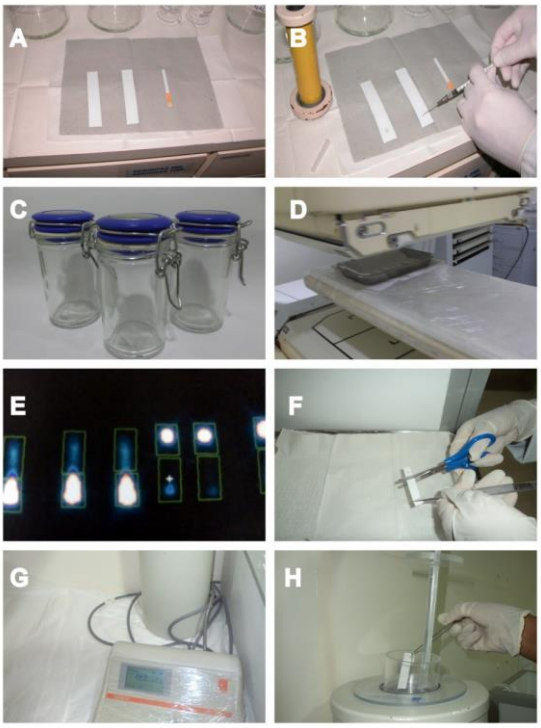

Figure 1: Procedure for the evaluation of the radiochemical purity using chromatographic strips at two different detection systems: scintillation chamber and the dose calibrator.

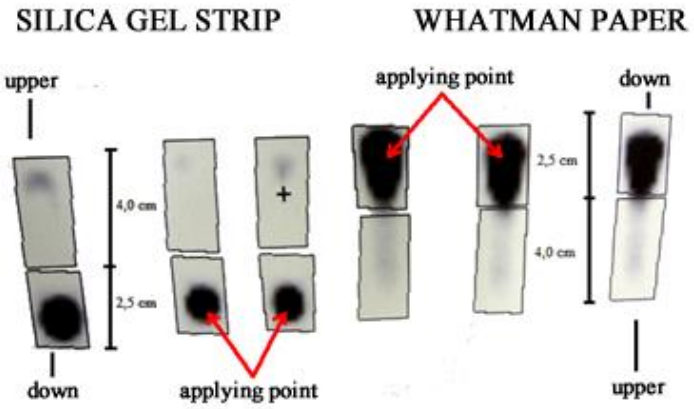

Figure 2: Selected images of ROIs on silica gel ribbons and Whatman paper to quantify the counts

In the second method, we used the dose calibrator of each NMS [5]. The ribbons were cut in half (Figure 1F) and the activity was determined using the ^{99m}Tc channel, as shown in Figure 1G and 1H.

The results obtained were compared with acceptable limit values of PRQ in the inserts of manufacturers, values higher than 90% [6; 7].

2.2. *In Vivo* Activity Quantification

For the *in vivo* activity quantification, it was used a technique that combines opposing planar images, also known as combined images counting technique. The values obtained with this method are theoretically independent on the depth of the tissue source [3; 8].

Two images of the chest/abdomen were acquired from each patient, one (front and rear) following the acquisition of cardiac imaging in stress condition. This helps to identify perfusion defects [8]. Twenty patients images were acquired for each one of the services A, B and C, 10 of these patients had radiopharmaceutical from Manufacturer 1 injected in their bodies and 10 from Manufacturer 2, using the following acquisition protocol: Collimator for low energy; Power window in 20%, centered in the photopeak; 256x256 acquisition matrix; acquisition time (5 minutes). The distance from the patient to the collimator was around 15 to 25 cm according to the thickness of each patient (Figure 3).

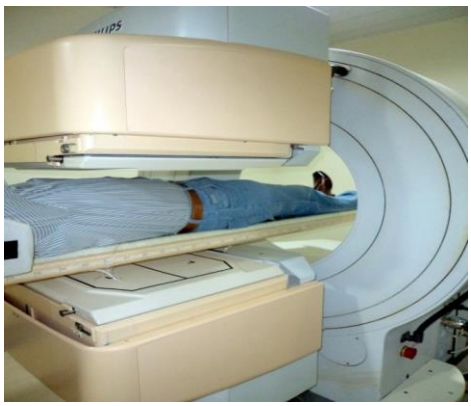

Figure 3: Patient positioning to acquire the image (front/rear).

To obtain the total counts in the organs of interest (heart and liver) were made ROIs contour, as shown in Figure 4. Each image was identified as Pn, that is the identification of the patient associated with the radioisotope used.

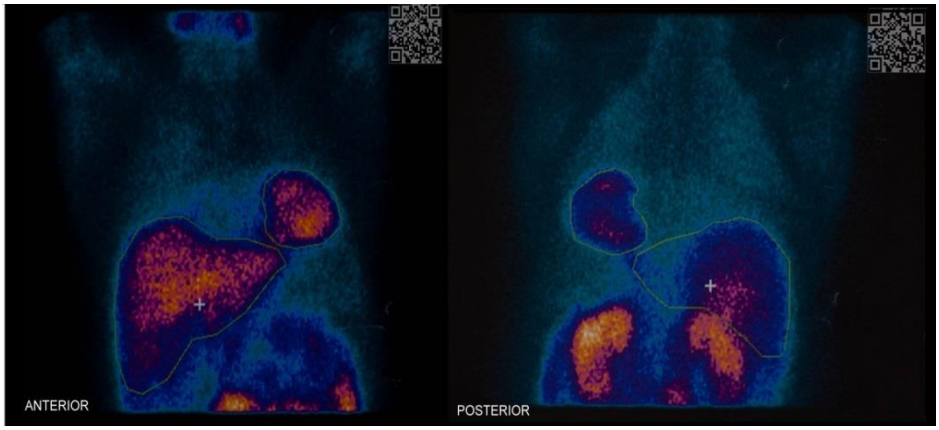

Figure 4: ROIs in the organs of interest (Heart/ Liver)

The NMS A, B and C have different protocols performing scintigraphy of the myocardium; these protocols are described below:

Service A

- One day protocol: first the patient is at rest and then at stress, or vice versa, with a given activity related to weight or even with activities related to the double compared to the first activity, which may vary from 296 MBq to 555 MBq (8 mCi to 15 mCi).
- Two days protocol: First the patient is at a one-day rest and then at stress on the following day. In this protocol it is injected into the patient the same activity which is from 740 MBq to 925 MBq (20 mCi to 25 mCi) independent on the phase (Rest or Stress). It is more commonly used in patients who weigh up to 100kg or those with a problem that could prevent the exam in a single day.

Service B

- One day protocol: First it is performed at rest and and activity around 444 MBq is managed (12 mCi). In the stress phase it is given an activity that is the triple of the first one, i.e. about 1332 MBq (36 mCi).
- Two days protocol: Similar to Service A.

Service C

This service has only one-day protocol. Starting from the stress phase, it is managed to the patient an activity of about 370 MBq (10 mCi). In the second step, the triple of the first one is administered.

In all NMS, theoretically, patients make the first image approximately one hour after the administration of the ^{99m} Tc-MIBI radiopharmaceutical in both stages of the test.

For this work the activity was quantified in the heart and the liver and the chosen configuration took into consideration that these organs are part of a region with a uniform distribution activity.

The activity of the source region, represented by A1, was obtained by the equation (2) [8]:

$$A_1 = F \cdot \sqrt{\frac{I_A \cdot I_p}{e^{-\mu x}}} \cdot \frac{f}{C} \quad (2)$$

where: I_A *and* I_P are counts rates per second (CPS) obtained in the previous and subsequent images, respectively, in each ROI; $e^{-\mu x}$ is the gamma rays transmission factor through the thickness (x) of the patient with the linear attenuation coefficient (μ); f is the factor that represents the correction for the self-attenuation of the source and as it involves only the characteristics of the source region, it is not significantly different from 1, which is the value considered for the solution of equation 2; C is the system calibration factor (count rate per unit of activity). This factor was obtained by performing counting in a known activity for a established period of time in a standard geometry and air.

F - is a factor used to subtract the contribution from the activity of an area next to the region of interest on the activity of this region. If the source region is located in a region with a uniform distribution of activity, the fraction of the geometric mean of counts $(IA.IP)^{1/2}$ that is inserted in the region of interest is given by equation (3):

$$F = \left\{\left[1-\left(I_{adj}/I_A\right)\left(1-\left(t_j/t\right)\right)\right]\cdot\left[1-\left(I_{adj}/I_p\right)\left(1-\left(t_j/t\right)\right)\right]\right\}^{1/2} \quad (3)$$

where: I_{adj} is the count obtained from the patient to a region next to the organ with the same area; t represents the thickness of the patient; tj. represents the thickness of the region or source organ that can be obtained by the analysis of a side view of the patient or the use of standard values found in the existing literature. In this work, it was used the thickness of the 6cm to the heart and 12 cm to the liver [9].

3. RESULTS AND DISCUSSION

3.1 The ^{99m}Tc-MIBI Radiochemical Purity Assessment (PRQ)

The results of the ^{99m}Tc-MIBI radiochemical purity in clinics A, B and C for the dose calibrator and the scintillation chamber are shown in Tables 1 and 2.

It is noted that for clinic A, the two manufacturers showed mean percentage of radiochemical purity higher than 90% using both methods. In clinic B, only a sample from Manufacturer 2 presented results lower than 90% in the dose calibrator. And clinic C, samples from both manufacturers using the method of the scintillation chamber, showed average higher than 90%, but when using the same samples in the dose calibrator, the results presented were lower than the limit of acceptability.

The determination of the percentage of PRQ appeared to be more convenient using the scintillation chamber, because it proves to be more sensitive in the analysis of the chromatographic strips. This was also observed by Melo [4].

Table 1: Purity percentage values of radiochemical of the ^{99m}Tc-MIBI at NMS A, B and C obtained by the dose calibrator.

Service	Mean Percentage of Purity Radiochemical (%)		Mean ± Standard Deviation (%)	
	Manufacturer 1	Manufacturer 2	Manufacture1	Manufacturer 2
A	Sample 1 = 98 Sample 2 = 97	Sample 1 = 97 Sample 2 = 98	97,5 ± 0,5	97,5 ± 0,5
B	Sample 1 = 83 Sample 2 = 96 Sample 3 = 91	Sample 1 = 95 Sample 2 = 90 Sample 3 = 78	90 ± 5	87 ± 7
C	Sample 1 = 72 Sample 2 = 82 Sample 3 = 75 Sample 4 = 75	Sample 1 = 86 Sample 2 = 93 Sample 3 = 64 Sample 4 = 77	76 ± 3	80 ± 10

Table 2: Purity percentage values of radiochemical of the ^{99m}Tc-MIBI at NMS A, B and C obtained by the scintillation chamber.

Service	Mean Percentage of Purity Radiochemical (%)		Mean ± Standard Deviation (%)	
	Manufacturer 1	Manufacturer 2	Manufacturer 1	Manufacturer 2
A	Sample 1 = 98 Sample 2 = 99	Sample 1 = 98 Sample 2 = 97	98,5 ± 0,5	97,5 ±0,5
B	Sample 1 = 98 Sample 2 = 99 Sample 3 = 99	Sample 1 = 97 Sample 2 = 99 Sample 3 = 73	98,6 ± 0,5	90 ± 12
C	Sample 1 = 83 Sample 2 = 93 Sample 3 = 95 Sample 4 = 95	Sample 1 = 85 Sample 2 = 89 Sample 3 = 98 Sample 4 = 99	91 ± 5	93 ± 6

However, clinic A showed the same PRQ values percentage with both methods. And this NMS is the only one performing quality control of the dose calibrator daily, this may be one of the reasons that the radiochemical purity values observed in other clinics showed results below the limits recommended by the pharmacopoeia, when the measurements were performed with such equipment.

3.2 Quantification of Activity "In Vivo"

In order to compare the results observed of the activities quantified in heart and liver of patients at services A, B and C, the specific activity was used. For this, data of a standard male (1.70m high and 70kg) were considered, such as the mass values of 330g and 1800g for heart and liver, respectively [10]. The results are presented in terms of specific activity (MBq/g) in Tables 3, 4 and 5.

Table 3: Quantification of liver/ heart activity in patients at service A

Patient	PRQ (%)	Administered Activity in stress (MBq)	Time Injection/ Image (min)	Specifies Activity (MBq/g)		Ratio Heart/Live
				Heart	Liver	
1	97	370	70	0,0231	0,0307	1,329
2	97	370	79	0,0170	0,0097	0,572
3	97	370	65	0,0206	0,0236	1,148
4	97	296	61	0,0084	0,0135	1,608
5	97	370	123	0,0267	0,0075	0,281
6	98	370	68	0,0440	0,0251	0,570
7	98	370	81	0,0219	0,0039	0,178
8	98	444	105	0,0384	0,0224	0,583
9	98	370	70	0,0209	0,0006	0,029
10	98	370	89	0,0218	0,0126	0,577
11	97	407	92	0,0346	0,0054	0,156
12	97	518	67	0,0169	0,0273	1,615
13	97	666	70	0,0180	0,0136	0,756
14	97	1258	93	0,0569	0,0121	0,213
15	97	444	125	0,0198	0,0021	0,108
16	97	555	120	0,0081	0,0069	0,844
17	98	1332	62	0,0841	0,0597	0,710
18	98	888	79	0,0416	0,0367	0,883
19	98	888	95	0,0507	0,0002	0,005
20	98	481	83	0,0081	0,007	0,864
Mean	97,5±0,5	557±301	85±20	0,029±0,019	0,016±0,015	0,651±0,480

It is observed by the data in Table 3 that at service A, all the radiopharmaceutical samples used had a good percentage of radiochemical purity (≥97%) and the average specific activity of the heart was 0.029 MBq/g. It is important to highlight that four patients (Pn 14, Pn 17, Pn Pn 18 and 19) performed the first resting phase, probably present higher specific activities in heart. In service B, there was a label problem in one of the sestamibi kits, with a percentage of 73% PRQ. As can be seen in Table 4, the average specific activity of the heart is lower than that one of service A (0.023 MBq/g). However this is not due exclusively to poor labelling of that kit, but also the average activity administered to the patient. The average specific activity of the heart, excluding those patients who received the radiopharmaceutical with a low radiochemical purity, was 0.021 ± 0.013 MBq/g.

The data in Table 5 show that, in service C, three kits presented percentages of PRQ lower than the limit set by the manufacturers. Dividing then in two groups, PRQ <90% and ≥ 90% PRQ, the average specific activities of the heart were 0.018 MBq/g and 0,028MBq/g, respectively. It is noted that the average value for kits with labelling problems was lower, demonstrating quantitatively that when the percentage of radiopharmaceutical has PRQ lower than the expected, one can obtain images with lower scores statistics. Here it is the importance of this test to be performed before the administration to the patient.

Table 4: Quantification of liver/ heart activity in patients at service B

Patient	PRQ (%)	Administered Activity in Stress (MBq)	Time injection/ image (min)	Specifies Activity (MBq/g)		Ratio Heart/Liver
				Heart	Live	
1	97	444	60	0,017	0,003	0,161
2	97	444	105	0,013	0,006	0,440
3	97	444	115	0,022	0,007	0,340
4	99	444	110	0,018	0,004	0,214
5	99	444	150	0,011	0,007	0,697
6	99	444	60	0,017	0,022	1,252
7	73	444	15	0,027	0,024	0,883
8	73	444	75	0,013	0,007	0,518
9	73	444	45	0,061	0,018	0,303
10	73	444	110	0,020	0,012	0,608
11	98	444	60	0,040	0,013	0,319
12	98	444	60	0,011	0,002	0,188
13	98	444	60	0,012	0,004	0,358
14	99	444	60	0,018	0,010	0,564
15	99	444	60	0,009	0,006	0,605
16	99	444	60	0,014	0,014	0,968
17	99	444	60	0,024	0,043	1,785
18	99	444	100	0,016	0,014	0,868
19	99	444	65	0,057	0,032	0,570
20	99	444	55	0,040	0,007	0,156
Mean	93±10	444	74±31	0,023±0,015	0,013±0,011	0,590±0,397

However, comparing the average of this group (PRQ <90%) with individual values of patients who received ^{99m}Tc-MIBI radiopharmaceutical with PRQ ≥ 90%, there are equivalent values. This fact shows that there are other factors beyond the radiochemical purity that may be influencing the radiotracer uptake in the heart, such as coronary flow of the individual [11]. This was also observed by Pereira who suggests that it is related to the physicochemical characteristics of the patient [12].

The liver activities were quantified for this is an organ that secretes the ^{99m} Tc-MIBI radiopharmaceutical, together with bile salts, and for being very close to the heart. If there is a high activity in that organ, due to proximity to this, myocardial scintigraphy may be aggrieved [13]. And, according to Mannting et al, when the ratio of liver/heart activity has a value higher than one at the time of the image acquisition, generated reconstruction artifacts can be at the time of the image processing, complicating medical reports [14].

From Tables 3 to 5, it is observed that the average specific activity of the liver was higher at service A(0.013 MBq/g), where it was also administered an average activity higher than 557 MBq (15mCi). It is also worthy to mention that the stress phase in most of the patients (85%) was performed using dipyridamole (exclusively pharmacological stress), which may have influenced the slower elimination of the liver and organs of the gastric system [12;14]. In the case of services B and C, where the averages were lower, 45% of patients had exclusive

pharmacological stress. Even in these cases, the British Cardiac Society recommends that the images to be carried out 60 minutes after the stress. On the other hand, the American Heart Association recommends 45 minutes [15].

Table 5: Quantification of liver/ heart activity in patients at service C

Patient	PRQ (%)	Administered Activity in stress (MBq)	Time injection/ Image (min)	Specifies Activity (MBq/g)		Ratio Heart/Live
				Heart	Live	
1	85	370	150	0,0343	0,0096	0,278
2	85	370	100	0,0145	0,0052	0,357
3	89	370	63	0,0169	0,0085	0,504
4	89	370	90	0,0156	0,0009	0,060
5	89	370	85	0,0124	0,0001	0,005
6	98	296	95	0,0084	0,0113	1,346
7	98	296	60	0,0064	0,0040	0,624
8	98	296	40	0,0092	0,0059	0,639
9	98	296	50	0,0132	0,0058	0,437
10	98	370	96	0,0082	0,0018	0,216
11	98	370	46	0,0111	0,0101	0,910
12	83	370	65	0,0248	0,0031	0,125
13	83	370	75	0,0158	0,0066	0,419
14	83	370	75	0,0078	0,0011	0,144
15	93	370	47	0,0486	0,0061	0,125
16	93	1110	65	0,0695	0,0128	0,184
17	94	1110	60	0,0975	0,0549	0,563
18	94	1110	41	0,0208	0,0122	0,587
19	94	1110	74	0,0209	0,0026	0,124
20	95	370	60	0,0127	0,0131	1,026
Mean	91±6	503±313	72±26	0,030±0,038	0,009±0,012	0,433±0,344

Regarding the quantification of activity in liver/heart proportion, service A presented four images with an aspect ratio higher than 1. Services B and C presented two images each. Despite service C had presented percentages of PRQ lower than 90% in 3 labelled kits, it also presented an average liver/heart proportion lower than the other services. This seems to show that the liver/heart proportion is not a factor that depends on the labeling efficiency.

No differences were observed in the values of the specific activities (heart and liver) and therefore in the relation liver/heart, among manufacturers, as well as radiochemical purity, demonstrating to be no problem of a drug vendor.

4. CONCLUSION

There is the need to implement a quality control of radiopharmaceuticals program, since the results demonstrated the possibility of obtaining samples of ^{99m}Tc-MIBI radiopharmaceutical,

with specifications of non-compliant quality assurance with the benchmarks set out by international pharmacopoeia and by manufacturers.

It is necessary to keep the dose calibrator as quality controls up to date in order to perform radiopharmaceuticals quality tests in an efficiently way. Also it is suggested the method with the scintillation chamber as an option to confirm the results obtained as the dose calibrator that show values lower than the limits set by pharmacopoeias and manufacturers.

It was shown that the quality control of ^{99m}Tc-MIBI radiopharmaceutical, regardless the manufacturer, did not affect the result of the value of quantified activity in the heart and liver through their image.

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