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DOSE CALIBRATOR AND GAMMA COUNTER: COMPARISON OF RESULTS IN MIBI-TEC® BIOLOGICAL DISTRIBUTION

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ABSTRACT

To ensure that pharmaceutical products have and maintain the structure, identity, purity, concentration, potency and safety characteristics required for their use, there is a set of standard procedures called Good Manufacturing Practices (GMP). In Brazil, the National Sanitary Surveillance Agency (ANVISA) regulates the GMP production of medicines through RDC17/2010 and for radiopharmaceuticals through RDC 63/2009 and 38/2008, to ensure their safe and correct use in commercial production and nuclear medicine services, respectively. Most ^{99m}Tc radiopharmaceutical monographs have biological distribution specifications. Using invasive method, ^{99m}Tcradiopharmaceuticalsare assessed by the injection into animals of defined strains and the radioactivity (as percentage of retainedor injected dose -%RDor%ID)ismeasured in specified organs. Technetium-99m Sestamibi radiopharmaceutical (^{99m}Tc-2-methoxy-isobutyl-isonitrile; ^{99m}Tc-sestamibi; MIBI-TEC®) monograph is related in USP 41, but the biological distribution assay is not included. At IPEN, the biodistribution test is performed as established in the Radiopharmaceutical Quality Control Manual of ARCAL XV (1999) - International Atomic Energy Agency (IAEA), for each batch. The objective of this work is to compare MIBI-TEC®biodistribution results using dose calibrator and gamma counter with sodium iodide detector by measuring the radioactivity in the organs. Three batches of MIBI-TEC®were used and 1 vial of lyophilized reagent (LR) was labeled with 5-10 mCiin 1-3 mL of ^{99m}Tc eluate. 200-300 µCi in 0.2 mLwereinjected in 3 *Balb-C*mice. After 30 minutes of biodistribution, heart, lung, muscle, liver, paw, column and tail were withdrawn, weighed and radioactivity was measured in dose calibrator and gamma counter (µCi andcpm, respectively). %ID/g ratio in the organs of interest was calculated using the data obtained by both equipment and the results were compared. No significant differences were observed and it was possible to conclude that either a dose calibrator or a gamma counter can be used in the routine of quality control.

1. INTRODUCTION

To ensure that pharmaceutical products have and maintain the structure, identity, purity, concentration, potency and safety characteristics required for their use, there is a set of standard procedures called Good Manufacturing Practices (GMP) [1].

In Brazil, the National Sanitary Surveillance Agency (ANVISA) regulates the GMP production of medicines through RDC 17/2010. Radiopharmaceuticals require specialized techniques in their handling and are regulated by RDC 63/2009 and 38/2008, which are intended to ensure that these products have and maintain the necessary characteristics for their safe and correct use in commercial production and Nuclear Medicine services, respectively [1, 2, 3].

Most ^{99m}Tc radiopharmaceutical monographs in the main international pharmacopoeias (American, European and International) and also in the Brazilian pharmacopoeia have biological distribution specifications. These results and other related to the radiochemical purity (RCP), bacterial endotoxins and sterility test are the main part of the documentation to release a radiopharmaceutical batch [4, 5, 6, 7].

Using invasive method,a^{99m}Tc radiopharmaceutical has to be injected into animals of defined strains and the measured radioactivity (as percentage of retained or injected dose – %RD or %ID) in specified organsis evaluated according to the formulation and application of each radiopharmaceutical. For each radiopharmaceutical, theinternational pharmacopeia monographs establish limits for some organs.

Technetium-99m Sestamibi radiopharmaceutical (^{99m}Tc-2-methoxy-isobutyl-isonitrile; ^{99m}Tc-sestamibi; MIBI-TEC®) is a small, lipophilic and cationic compound used for myocardial perfusion imaging. In addition, ^{99m}Tc-MIBI has been shown to be useful in identifying several types of tumors, such as breast, lung and thyroid cancers [8].

At IPEN, the MIBI-TEC® biodistribution test is performed in accordance with the Radiopharmaceutical Quality Control Manual - ARCAL XV (1999) of the International Atomic Energy Agency (IAEA).^{99m}Tc-MIBI monograph is related in USP 41 and EP 9.0, but the biological distribution assay is not included [4, 5, 9].

One of the most commonly used equipment in nuclear medicine services to measure radioactive samples is the dose calibrator. The radioactivity is measured as ionizations produced within the well-type ionization chamber as multiples or submultiples of Curie or Becquerel units [10].

The gamma counter is widely used in measurements for RCP determination in research and development laboratories and in industrial radiopharmacy. The well-type gamma counter consists of a system that allows the insertion of samples into structures called wells, placed next to a thallium doped sodium iodide [NaI (Tl)]crystal coupled to a photomultiplier. Each well (detection unit) has a lead shield so that the influence of radiation on one well is minimal on adjacent wells. The gamma radiation present in the sample interacts with the crystal generating light photons (scintillation) and these events are processed and accounted for thereby obtaining the sample counts [10].

The objective of this work is to compare MIBI-TEC® results using a dose calibrator and a gamma counterwith sodium iodide detectorby measuring the radioactivity in the organs of interest.

2. EXPERIMENTAL

Three batches of MIBI-TEC® were analyzed using 3 *Balb-C*mice (20-25 g weight) for each batch. The lyophilized cold kit was labeled with 370 MBq (10 mCi) in 3 mL $Na^{99m}TcO_4$ solution. After 10 minutes of 100°C heating and 20 minutes of cooling at room temperature, a dose of 7400-11100 MBq (200-300 μCi) in 0.2 mL was administered into the caudal vein of each animal. About 30 minutes after injection, the animals were euthanized with a

lethalanesthetic dose and samples of heart, lung, muscle, liver, paw and column (to calculate body weight) and tail were withdrawn and weighed (Micronal PB 303Analytical Balance).

The radioactivity of each organ was firstly measured in a dose calibrator (CRC-35R, Capintec Inc., EUA) and expressed asmCi and sequentially in a gamma counter with sodium iodide detector(Perkin Elmer Gamma Counter, Perkin Elmer)in ^{99m}Tc window of 70 – 210 keV energy during 0.20 minutes and expressed ascounts per minute (cpm).The percentage of injected dose (%I.D.) was calculated for the data obtainedin both equipment.

Thiswork was carried out according to the ethical standards required by the National Commission for Research Ethics / National Health Council / Ministry of Health (CONEP / CNS / MS).

3. RESULTS AND DISCUSSION

The biological distribution assay shouldroutinely be performed to evaluate the *in vivo* behavior of ^{99m}Tc radiopharmaceuticals prior tothe release of each batch. This assay, if quoted in officialmonographs, must also be included in the stability studiesof ^{99m}Tc radiopharmaceuticals to determine the cold kit shelf life. It is related to the RCP, since the presence of radiochemical impurities can induce alterations in the biodistribution of the radiopharmaceuticals [11].

IPEN-CNEN/SP has established the biological distribution control for MIBI-TEC® with ARCAL specification limits and validated its use by including the relevant documentation in the Product Registration Report to the regulatory authorities.

Not less than 2 of the 3 animals evaluated should meet the following specifications (Equations 1-3) [9]:

$$\frac{\%I.D./g\ Heart}{\%I.D./mL\ Blood} \geq 10 \quad (1)$$

$$\frac{\%I.D./g\ Heart}{\%I.D./g\ Lung} \geq 7 \quad (2)$$

$$\frac{\%I.D./g\ Heart}{\%I.D./g\ Muscle} \geq 4 \quad (3)$$

In the biological distribution assay, the dose calibrator is routinely used for the measurement of the radioactivity of heart, lung, muscle and blood using 11 x 4 cm diameter tubes, in which the whole organ can be easily introduced with a reduced risk of contamination, when compared with the 8 x 1.5 cm gamma counter tube where only a fraction of the organ can be counted.

Table 1 shows the results of biological distribution for three MIBI-TEC® batches obtained in a dose calibrator and a gamma counter with sodium iodide detector.

Table 1: Results of biological distribution for three MIBI-TEC® batches obtained in a dose calibrator and a gamma counter with sodium iodide detector (n=3)

	Batch 1		Batch 2		Batch 3	
	µCi(1)	**cpm(2)**	**µCi(1)**	**cpm(2)**	**µCi(1)**	**cpm(2)**
$\frac{\%I.D./g\ Heart}{\%I.D./g\ Lung}$	11.0 ± 0.1	9.3 ± 0.5	11.4 ± 0.7	9.6 ± 3.1	10.8 ± 1.5	8.3 ± 1.1
$\frac{\%I.D./g\ Heart}{\%I.D./g\ Muscle}$	8.0 ± 0.6	5.1 ± 0.4	5.4 ± 2.7	4.4 ± 1.9	6.2 ± 0.1	4.7 ± 0.6
$\frac{\%I.D./g\ Heart}{\%I.D./mL\ Blood}$	186.8 ± 87.3	36.5 ± 7.8	123.1 ± 34.0	29.6 ± 2.8	64.1 ± 3.1	80.8 ± 4.2

(1) Dose calibrator
(2) Gamma counter

Differently from the first generation of radiopharmaceuticals such as ^{99m}Tc-DTPA or ^{99m}Tc-Tin Colloid, in which the biodistribution evaluates the radioactivity in high uptake organs, for example, 85% in the kidneys and bladder, and 80% in the liver and spleen, respectively, in the case of ^{99m}Tc-MIBI, it is not possible to simulate a condition of myocardial infarction in small animals in order to assess heart perfusion. It is expected low uptake (in the range of µCi) in adjacent organs to the heart as lung, muscle and even in the blood, and because thisratio is calculated with low values of radioactivity, the resultsarestrongly influenced by variations in the measurements [11].

Radiopharmaceuticals are generally distributed nonuniformly in tissues. Such nonuniformities are observed over the entire range of spatial levels, affecting the biological response of tissues containing radioactivity. This fact may explain the variations among the uptake measurements [12]

The reason for the differences between the two procedure results can be mainly attributed to the efficiency of the equipment for low radioactivity measurements, influenced by variation of geometry and sample size, measurement time [13].

Maioli *et al*. in a radiochemical purity study for several ^{99m}Tc radiopharmaceuticals, used dose calibrator and gamma counter to compare the obtained data and they have found statistically similar labeling efficiency values [14].

4. CONCLUSIONS

The results of biological distribution and other related specifications in MIBI-TEC®USP monograph, such as radiochemical purity, bacterial endotoxins and sterility tests arenecessary and important to release a radiopharmaceutical batch to the market.

No significant differences were observed and it was possible to conclude that either a dose calibrator or a gamma counter can be used in the routine of quality control.

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