ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR – ABEN
2019International Nuclear Atlantic Conference - INAC 2019
Santos, SP, Brazil, October 21-25, 2019

Agência Brasileira do ISBN
ISBN 978-85-99141-08-3
9 788599 141083

RADIOCHEMICAL PURITY DETERMINATION OF TECHNETIUM ^{99m}Tc-SESTAMIBI BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Patricia de A. Martins, Neuza T. O. Fukumori, Natanael G. da Silva and Margareth M. N. Matsuda

Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP, Brazil
patyosborne@yahoo.com

ABSTRACT

The assessment of the extent of reversible or irreversible mitochondrial damage after myocardial ischemia is performed by obtaining myocardial perfusion SPECT-CT images of Technetium-99m Sestamibi radiopharmaceutical (^{99m}Tc-2-methoxy-isobutyl-isonitrile; ^{99m}Tc-sestamibi; ^{99m}Tc-MIBI). For quality control purposes, the monograph of the United States Pharmacopoeia (USP) was followed. The determination of the radiochemical purity of ^{99m}Tc-sestamibi involves the use of two chromatographic methods: thin layer chromatography in reverse phase and high performance liquid chromatography. This work aims to determine the radiochemical purity of ^{99m}Tc-sestamibi by the HPLC method described in USP. The analyses were performed on a Shimadzu liquid chromatography, LC-20AT model, consisting of two pumps, degasser, automatic sample injector, UV-visible detector and Bioscan radioactivity detector. The column used was µBondapack C18 (3.9 x 3 00 mm, 10 µm, Waters) and the mobile phase was a mixture of acetonitrile, 0.05 mol L^{-1} ammonium sulfate and methanol (20:35:45). 5 µL of sample (approximately 250 µCi) was injected with a 2 mL min^{-1} mobile phase flow. According to the USP monograph, the retention time for ^{99m}Tc-sestamibi is 5-10 minutes and for the ^{99m}Tc-pentamibidimethylvinylisonitrile impurity is 6-13 minutes. Not less than 90% of the total radioactivity must be present as ^{99m}Tc-sestamibi and not more than 5% as ^{99m}Tc-pentamibidimethylvinylisonitrile. For 12 analyzed batches of MIBI-TEC® produced at IPEN/CNEN-SP, the product presented a retention time of 7 minutes and the ^{99m}Tc-pentamibidimethylvinylisonitrile impurity formation was not observed.

1. INTRODUCTION

Technetium-99m-sestamibi (^{99m}Tc-2-methoxy-isobutyl-isonitrile; ^{99m}Tc-sestamibi; ^{99m}Tc-MIBI) is a small, lipophilic and cationic compound used for myocardial perfusion imaging. The molecular structure of ^{99m}Tc-MIBI consists of one ^{99m}Tc atom and six MIBI molecules (Fig.1). ^{99m}Tc-MIBI has been useful in identifying several types of tumors, such as breast, lung and thyroid cancers [1].

The *in vivo* use of radiopharmaceuticals requires, in the case of ^{99m}Tc radiopharmaceuticals, the radiochemical purity (RCP) testing to be carried out just before the administration to the patient, as they are usually labeled in the hospital using commercially available cold kits and generators [2-3].

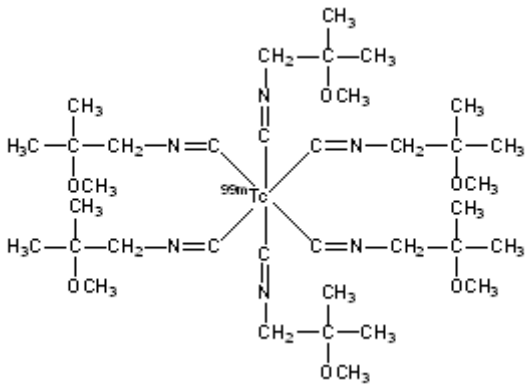

Figure 1: Structure of ^{99m}Tc-MIBI complex [4]

The methods used for quality control should be characterized by high accuracy and reliability, as well as being easy to perform, safe and quick in order to ensure the use in a busy laboratory or in emergency situations.

RCP which is the fraction of radioactivity present in the specified chemical form is frequently determined by chromatographic methods as thin layer chromatography (TLC), instant thin layer chromatography-silica gel (ITLC-SG), paper chromatography (PC), mini columns or high performance liquid chromatography (HPLC). These analytical techniques combined with radioactivity detection are the most important tool in the RCP determination of radiopharmaceutical compounds [5].

A quality control method described in the United States Pharmacopoeia (USP) and European Pharmacopeia monograph for ^{99m}Tc-MIBI establishes the use of two chromatographic methods: TLC in reverse phase and HPLC [6-7].

The main impurities of MIBI-TEC® labeling are $^{99m}TcO_4^-$, $^{99m}TcO_2$ and ^{99m}Tc-pentamibidimethylvinylisonitrile, in which case the third compound is the result of the reaction with five MIBI molecules. The HPLC performs the separation between the ^{99m}Tc-MIBI product and ^{99m}Tc-pentamibidimethylvinylisonitrile impurity that cannot be quantified in TLC system [1].

The USP methodology by TLC-RP uses a mixture of acetonitrile, methanol, 3.85% ammonium acetate, and tetrahydrofuran (4:3:2:1) as mobile phase. A mean of not less than 90% of the radioactivity is found at a Retention Factor (RF) value between 0.3 and 0.6. Free pertechnetate is located at RF about 0.8 to 1.0, and the radiocolloid is located at RF around 0.0 to 0.1. The sum of the mean percentages of free pertechnetate ($^{99m}TcO_4^-$), and colloid ^{99m}Tc ($^{99m}TcO_2$) shall not exceed 10% [6].

In the HPLC method, the retention time for ^{99m}Tc-sestamibi is 5-10 minutes and for ^{99m}Tc-pentamibidimethylvinylisonitrile impurity is 6-13 minutes. In the calculation of RCP, it is necessary to correct the presence of colloid, which is not measured by this method, taken by the Equation 1.

$$Cf = [(100\%) - (Ac)] / 100 \quad (1)$$

in which Cf is the correction factor, and Ac is the mean area percentage for the colloid obtained from the TLC-RP method. To obtain the corrected area percentage, it is necessary to multiply the correction factor (Cf) by the area percentage of the peaks present in the chromatogram.

Not less than 90% (corrected area percentage) of the total radioactivity is represented by ^{99m}Tc-sestamibi, and a mean of not more than 5% (corrected area percentage) of the total radioactivity is present as ^{99m}Tc-pentamibidimethylvinylisonitrile.

This work aims to determine the RCP of ^{99m}Tc-sestamibi by the HPLC method described in USP and its implementation in the Quality Control routine.

2. EXPERIMENTAL

Twelve batches of MIBI-TEC® lyophilized cold kit were labeled with 2 mL of 1850 MBq (50 mCi) of ^{99m}Tc in each vial as described in USP. The $^{99m}TcO_4^-$ eluate was obtained from a ^{99}Mo/^{99m}Tc generator weekly produced at IPEN-CNEN/SP. The analysis in HPLC was carried out 30 minutes after labeling, as determined by the manufacturer.

The HPLC system (LC 20AT Prominence) (Shimadzu, Japan) was composed by two pumps, auto sampler (SIL 20A), system controller (CBM 20A), diode array (SPD M20A), column oven (CTO 20A), UV-visible detector and radioactivity detector (Bioscan). Reagents from Merck-Millipore (Germany) were used to prepare the mobile phase. The analysis was performed using a Waters column µBondapack C18 (3.9 x 300 mm, 10 µm), a mixture of acetonitrile, 0.05mol L^{-1} ammonium sulfate and methanol (20:35:45) as the mobile phase, 2 mL min^{-1} of mobile phase flow and 5 µL of sample volume (approximately 250 µCi).

3. RESULTS AND DISCUSSION

The lyophilized cold kit MIBI-TEC® produced by IPEN-CNEN/SP contains tetrakis (2-MIBI) copper (I) tetrafluoroborate, stannous chloride dehydrate as reducing agent and other adjuvants such as cysteinehydrochloride monohydrate, sodium citrate, mannitol and the shelf life was determined as 12 months stored at 2-8 ºC temperature. The RCP is one of the quality control analyses to be performed in stability studies to determine the cold kit shelf life.

A RCP quality control method described in USP monograph for ^{99m}Tc-MIBI establishes the use of two chromatographic methods: TLC-RP and HPLC to determine the RCP of Technetium-99m-sestamibi. HPLC method complements the TLC-RP analysis, to quantify ^{99m}Tc-pentamibidimethylvinylisonitrile impurity.

In the HPLC method, MIBI-TEC® has to be prepared with at least 50 mCi (1850 MBq) of $Na^{99m}TcO_4$ to a vial, and the pH after reconstitution should be in the range of 5.0 and 6.0. Radiochemical purity was verified by TLC-RP with result greater than 90% labeling.

Figure 2 shows a $Na^{99m}TcO_4$ (370 MBq) chromatogram profile in the described HPLC system. $Na^{99m}TcO_4$ presented a single peak with a retention time of 1.6 minutes and represented the chromatogram profile of a not labeled cold kit.

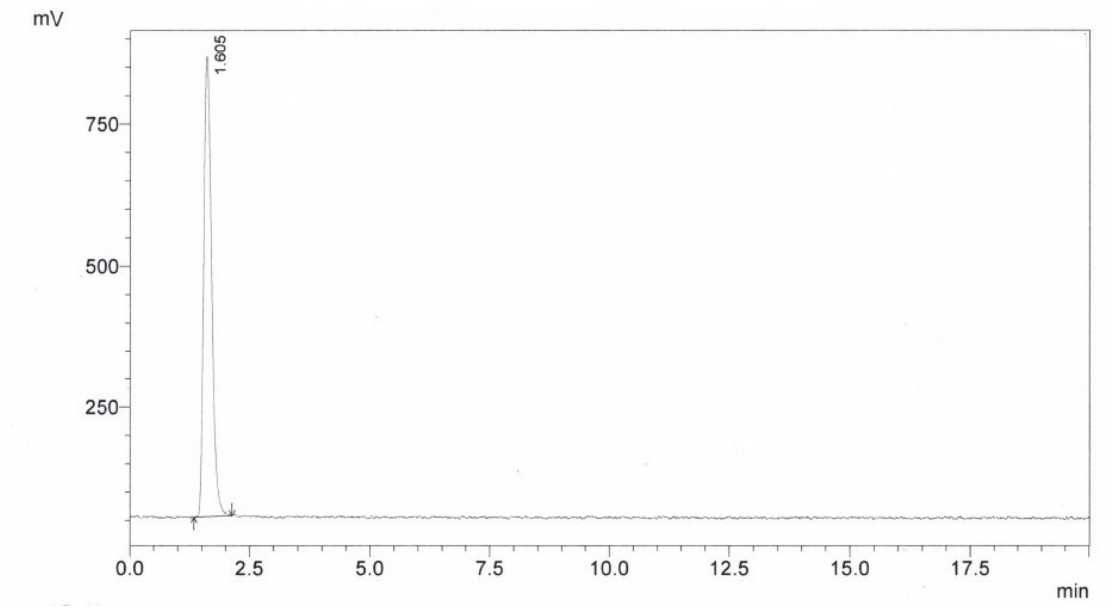

Figure 2: $Na^{99m}TcO_4$ chromatogram, 370 MBq (10 mCi) in the HPLC system using Waters column (3.9 x 300 mm, 10 μm); acetonitrile, 0.05 mol L^{-1} ammonium sulfate and methanol (20:35:45) mobile phase; 2 mL min^{-1} flow rate and 5 μL sample volume.

Figure 3 features the chromatogram profile and retention time of labeled MIBI-TEC® in the HPLC system.

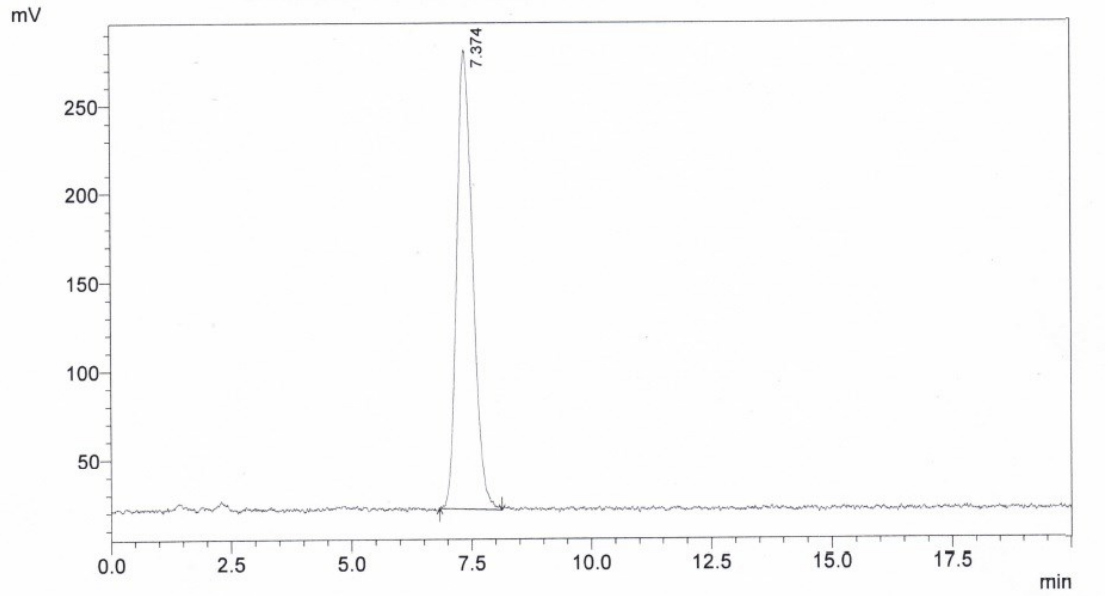

Figure 3: MIBI-TEC® chromatogram, 1850MBq (50 mCi) in the HPLC system using Waters column (3.9 x 300 mm, 10 μm); acetonitrile, 0.05 mol L^{-1} ammonium sulfate and methanol (20:35:45) mobile phase; 2 mL min^{-1} flow rate and 5 μL sample volume.

The methodology by HPLC was implemented with all the analysis conditions including the chromatographic column as described in USP. The column recommended in the monograph is composed by a stationary phase which is not used nowadays anymore, and produces a large peak.

Several factors may influence the labeling efficiency of the MIBI-TEC® kit, such as the amount of $Na^{99m}TcO_4$ activity added, the time and temperature of heating, and the age of the kit [1], but for the 12 analyzed batches of MIBI-TEC® the chromatogram profile showed a single peak in approximately 7.0 minutes, indicating that the analytical conditions in the USP monograph could not identify the impurity formation in the radiopharmaceutical produced at IPEN-CNEN/SP. The data obtained are similar to the data found in the literature [1].

In all batches the sum of mean percentages of free pertechnetate ($^{99m}TcO_4^-$) and ^{99m}Tc-colloid ($^{99m}TcO_2$) did not exceed 10% in TLC-RP, and after using the Equation 1, the RCP were above 90%.

4. CONCLUSIONS

In this work it was possible to confirm the high quality of MIBI-TEC® produced at IPEN-CNEN/SP which was proven by the successful results that enabled the implementation of the method by HPLC in the quality control routine, complementing the TLC-RP system for determination of RCP. No formation of the ^{99m}Tc-pentamibidimethylvinylisonitrile impurity was observed.

REFERENCES

1. B. Tasdelen. “HPLC investigation of the radiochemical purity of ^{99m}Tc-MIBI”, *J Radioanal. Nucl. Chem.*, **290**, pp. 283-287 (2011).
2. C. Maioli, A. Bestetti, F. Milani, G. P. Cornalba, L. Tagliabue, D. Di Benedetto, I. Rognoni, G. L. Tarolo, R. Paroni, "Evaluation of Different Counting Methods for Use in Radiochemical Purity Testing Procedures for ^{99m}Tc-labelled Radiopharmaceuticals", *Appl. Radiat. Isot*, **66**, pp. 556-559 (2008).
3. ANVISA. Agencia Nacional de Vigilância Sanitária. Resolução da diretoria colegiada – RDC nº 38, de 4 de Julho de 2008. Disponível em:< www.anvisa.gov.br/legis> acesso em: 04 jul 2019.
4. Disponível em: <http://www.druglead.com/cds/Technetium-99mTc-Sestamibi.html>, acesso em 04 jul 2019.
5. C. Decristoforo, R. Siller, F. Chen, G. Riccabona, " Radiochemical Purity of Routinely Prepared ^{99m}Tc Radiopharmaceuticals: A Retrospective Study", *Nucl. Med. Comm.*, **21**, pp. 349-354 (2000).
6. *United States Pharmacopeia 41*, United States Pharmacopeial Convention, Rockville, United States (2019).
7. *European Pharmacopeia 9.0*, EDQM Council of Europe, Strasbourg, France (2017).