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Villas Boas, Cristian Antonio Wieczorek; Pereira Dias, Luís Alberto; Nakamura Matsuda, Margareth Mie; Bortoleti de Araújo, Elaine
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Stability in production and transport of ^{177}Lu labeled PSMA

Villas Boas[a] C.A.W., Dias[a] L.A.P., Matsuda[a] M.M.N., Araújo[a] E.B.

[a] *Nuclear and Energy Research Institute/Radiopharmacy Center, 2242 Professor Lineu Prestes Avenue, University City, São Paulo 05508-000, Brazil*

cristianwvboas@gmail.com

ABSTRACT

In Brazil, prostate cancer is a common disease among men. The radionuclide therapy with PSMA analogs, labeled with beta and alpha emitters, has brought new perspectives for patients with multi metastatic resistant prostate cancer. The commercialization of radiopharmaceutical becomes a challenge to transport and ensure the quality of the product, especially in terms of radiochemical stability of therapeutic radiopharmaceuticals. The effect of radiolysis on therapeutic doses of radiopharmaceuticals can be avoided or mitigated by diluting the final dose and reducing storage and transportation temperature. This study evaluated the effect of radiolysis on the different radioactive concentration in the industrial batches of 177Lu-PSMA-617 and in the fractionated doses, considering long time transportation. The radiopharmaceutical 177Lu-PSMA-617 was produced in batches reaching 37 GBq, and stability studies were performed in controlled conditions. The results showed that a combination of factors, including reaction buffer, the radioactive concentration of final product, and freezing storage contributed to the stability of the radiopharmaceutical for 48 hours, enabling transport of 177Lu-PSMA-617 to distant regions of the country.

Keywords: **^{177}Lu-PSMA-617, radiolysis, stability.**

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1. INTRODUCTION

In Brazil, just like in other countries, prostate cancer is a common disease among men. According to INCA (National Institute of Cancer - Brazil), prostate cancer is the second most prevalent disease, and more than 68,000 new cases were estimated in 2020 [1]. The PSMA therapy has brought new perspectives for patients with multi metastatic resistant prostate cancer.

The Nuclear and Energy Research Institute of the National Nuclear Energy Commission (IPEN-CNEN, São Paulo/Brazil) is an industrial radiopharmacy center that produces radiopharmaceuticals for therapy in batches for large distribution. IPEN currently produces at least six batches of the radiopharmaceutical ^{177}Lu-DOTATATE (111 GBq per each batch) per month for therapy of patients with neuroendocrine tumors, corresponding to the distribution of up to 54 doses of this radiopharmaceutical.

Considering the continental dimension of Brazil (8.5 million km^2), radiopharmaceutical distribution becomes a challenge due to transportation issues that might compromise the quality of the product especially in terms of radiochemical stability of therapeutic radiopharmaceuticals.

The ready-to-use radiopharmaceuticals, especially for therapy, are subject to radiolysis and may lose affinity for the specific receptors. The β-particles emitted by ^{177}Lu in water produce reactive free radicals that can degrade radiopharmaceuticals molecules or chelators. The addition of quenchers, such as ascorbic acid, is an alternative to avoid the radiolysis effect and works as antioxidants [2,3].

Besides this, the effect of radiolysis on therapeutic doses of radiopharmaceuticals can be avoided or mitigated by diluting the final dose and reducing the temperature for storage and transportation [4].

To facilitate the distribution of therapeutic radiopharmaceuticals and, thereby, enable the development of clinical trials with ^{177}Lu-PSMA-617 in Brazil, this study evaluated the effect of radiolysis on the scale up to produce industrial batches of this radiopharmaceutical and on the fractionated doses for transport.

2. MATERIALS AND METHODS

2.1. Stability study of 740 MBq ^{177}Lu-PSMA-617 stored at room temperature and freezing conditions

The radiolabeling of PSMA-617 (ABX, Germany) was performed in a heating block for micro-tubes with 10 µg of PSMA-617, 100 µL of 0.52 M ascorbate buffer pH 4.7 and 740 MBq of $^{177}LuCl_3$ (INM, Russia) for 30 minutes at 90 ºC. After partial cooling of the microtube, 900 µL of 0.52 M ascorbate buffer pH 4.7 was added. The stability of this radiopharmaceutical (stored at room temperature and under freezing, -20 ºC) was checked immediately, after 24 hours and 48 hours. This assay was performed in triplicate.

2.2. Preparation of ^{177}Lu-PSMA-617

The radiolabeling of PSMA-617 was performed in a hot cell equipped with a heating block and a semi-automatic dispenser. The activity of $^{177}LuCl_3$ (NTP, South Africa, and INM, Russia; ≥ 3.000 GBq/mg) was increased for each produced batch reaching 7.4 GBq, 9.25 GBq, 18.5 GBq, 25.9 GBq, and 37 GBq, corresponding to 1 dose for immediate use, and 1, 2, 3 and 4 doses calibrated for use after 48 hours of production, keeping the specific activity constant (74 MBq/µg). The PSMA-617 was diluted in 0.52 M sodium ascorbate buffer pH 4.7, and the final radioactivity concentration did not exceed 3.7 GBq/mL. The reaction proceeded for 30 minutes at 90 ºC. After partial cooling of the reaction vial, 0.5 mL of DTPA solution (4 mg/mL DTPA in saline) was added, the volume was diluted with sterile saline solution, and the preparation was filter-sterilized (0.22 µm). The radiolabeled product was dispensed into 7.4 GBq doses, calibrated for 48 hours, and conditioned in appropriated lead chambers inside a dry ice bucket for transport simulation (Figure 1). Radiochemical purity (RCP) was determined immediately after radiolabeling by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The radionuclidic purity (%RNP), sterility, and bacterial endotoxins were also evaluated.

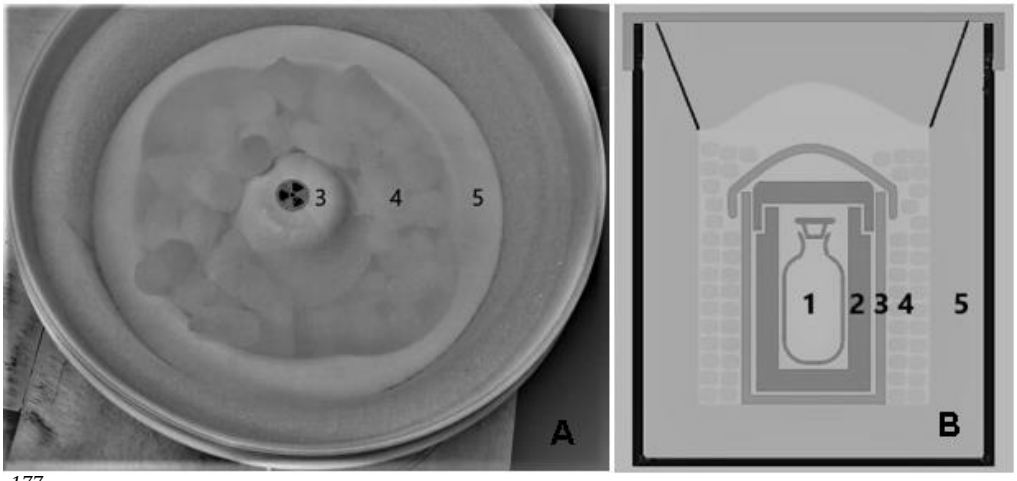

Figure 1. *^{177}Lu-PSMA-617 shipping packaging [A] and schematic representation [B]: vial [1] packaged under appropriated lead chamber [2] (intermediate packaging), plastic packaging with safety lock (secondary packaging) [3], and bucket [5] (transport packaging) with dry ice [4].*

2.3. Stability study of therapeutics doses

The stability of the ^{177}Lu-PSMA-617 was analyzed three times based on the percentage of radiochemical purity (%RCP), using two chromatographic procedures: immediately after radiolabeling, 24 hours later, and 48 hours the procedure. TLC analysis was performed using silica gel 60 plate (Merck, Germany) eluted using 0.1 M citrate buffer pH 5.0 as mobile phase. In this system the radiolabeled compound showed Rf 0.1-0.2 and the free lutetium-177 showed Rf 0.9-1.0. Radio-HPLC (Prominence, Shimadzu) analysis was performed using a C-18 column (Waters model Xterra RP 18.5 µm, 4.6 x 150 mm), eluted at flow rate of 0.4 mL/minute, by (A) water 0.1 % TFA and (B) acetonitrile, in gradient mode (0-2 min 17% B; 2.01-5.99 min 30% B; 6-20 min 17% B). In this system, the lutetium-177 was eluted with approximately 4.3 minutes and the ^{177}Lu-PSMA-617 with approximately 12.17 minutes.

2.4. Microbiological study

Sterility and endotoxin assays were performed in all batches, according to European Pharmacopoeia procedures. In the sterility assay, a diluted sample of ^{177}Lu-PSMA-617 was incubated in thioglycollate and trypticase soy broth medium at 32.5 ± 2.5 ºC and 22.5 ± 2.5 ºC, respectively, for 14 days.

The bacterial endotoxin test was performed using LAL (Limulus Amebocyte Lysate) gel clot test. A diluted sample of ^{177}Lu-PSMA-617 was added into a tube with 0.1 mL of LAL. The tube was incubated at 37 ± 1 ºC for 60 ± 2 minutes.

2.5. Radionuclidic purity

The radionuclidic purity, expressed as a percentage of the total radioactivity, was determined in gamma spectroscopy using a high-purity germanium (HPGe) detector in conjunction with a multi-channel analyzer (MCA).

3. RESULTS AND DISCUSSION

International publications rarely observe the aspect of stability in articles describing the preparation of radiopharmaceuticals for therapy. In most countries, peptide-based radiopharmaceuticals are prepared under hospital or centralized radiopharmacies, requiring no transport over long distances, to Nuclear Medicine centers. For instance, in 2019, researchers demonstrated that ^{177}Lu-PSMA-617 was stable for 24 hours at room temperature, but only when using a low activity such as 80 MBq [5]. In this work, the effect of the temperature on the stability for ^{177}Lu-PSMA-617 was evaluated using activity of 740 MBq, and the results showed that the product stored at room temperature was not stable, since the RCP decreased from around 98% to 80.4% ± 6.3% (n=3) after 24 hours. However, when the radiopharmaceutical was kept frozen (-20 ºC), the RCP was higher than 95% after 48 hours (figure 2). Based on these results, freezing was considered an option to study the stability for transporting therapeutic doses of radiopharmaceutical, since IPEN, located in São Paulo, produces and distributes therapeutic radiopharmaceuticals all over the country.

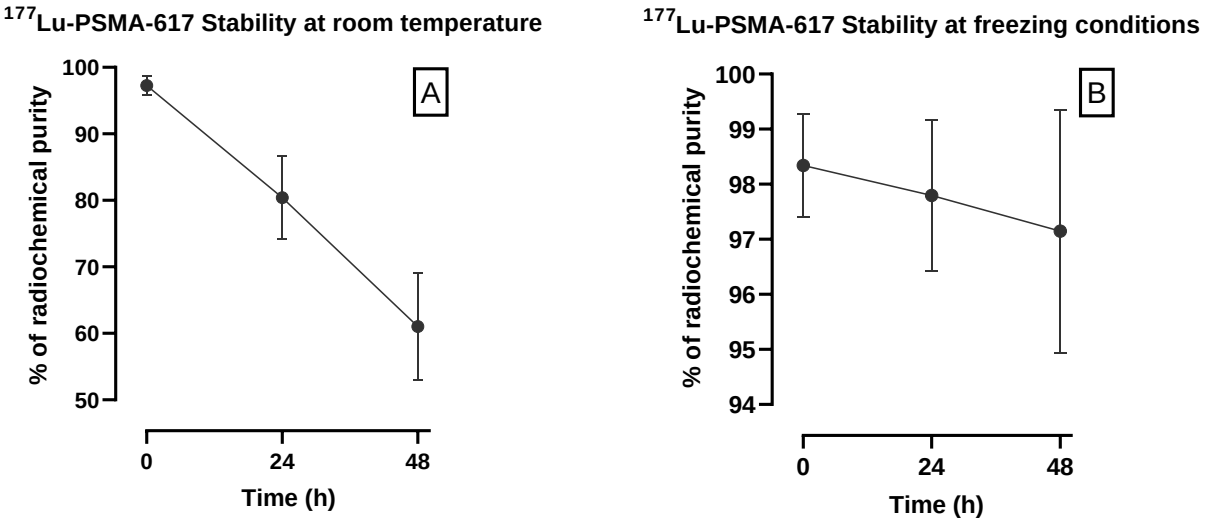

Figure 2. Stability studies of *^{177}Lu-PSMA-617 stored at room temperature [A] and freezing conditions (- 20 ºC) [B] (n=3).*

The batches produced for therapeutic doses were diluted to assure that the final concentration did not exceed 3.7 GBq/mL. The final volumes and concentrations are summarized in Table 1.

Table 1. Radiolabeling conditions and characteristic of the radiopharmaceutical ^{177}Lu-PSMA-617

Activity of lutetium-177 (GBq)	**PSMA mass (µg)**	**Buffer volume used in radiolabeling (mL)**	**Final volume after dilution with NaCl 0.9 % and sterile filtration (mL)**	**Final radioactive concentration (GBq/mL)**	**Approximate volume of 1 dose with 7.4 GBq* (mL)**
7.4	100	2.0	5.0	1.48	5.0
9.25	125	2.0	5.0	1.85	5.0
18.5	250	3.0	10.0	1.85	5.0
25.9	350	4.0	10.0	2.59	3.3
37.0	500	5.0	10.0	3.7	2.5

* Activity calibrated for 48 hours.

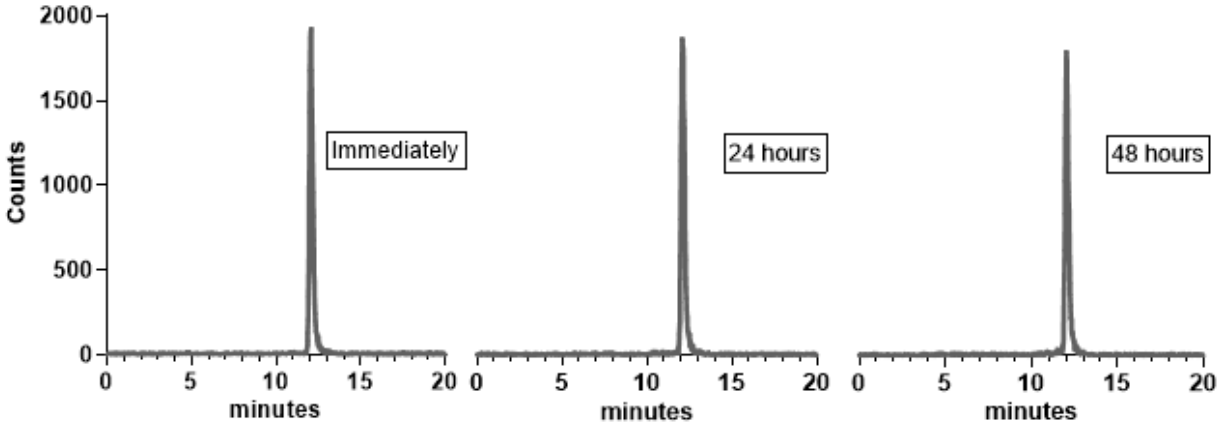

Figure 3. *HPLC profile of the ^{177}Lu-PSMA-617 therapeutic doses (7.4 GBq calibrated for 48 hours), obtained from the 37 GBq production batch and kept frozen for 24 and 48 hours. The radiochemical purity was 98.9 % and 96.8 %, respectively, proving the stability. The retention time was 12.21 minutes, 12.25 minutes, and 12.26 minutes for the radiochromatograms obtained immediately, 24 hours and 48 hours, respectively.*

All batches were produced according to the Brazilian Resolution [6] for GMP (good manufacture practices), meeting all acceptance criteria established for the performed tests. The batches were released for transportation after checking all the production records and the results of available quality controls assays (radiochemical purity and pyrogen). The therapeutic doses were sent to a hospital located 420 km away from the producing center, for use in compassionate clinical studies.

Radionuclidic purity assays demonstrated that the lutetium-177 from both suppliers (NTP, South Africa and INM, Russia) was more than 99.9% pure in the total activity. In addition, no traces of ytterbium-175, lutetium-177 metastable, or any other radioisotope as a contaminant were founded.

The therapeutic doses were stable for 48 hours (%RCP $\geq$ 95%), under freezing, for all studied radiolabeling conditions, final radioactive concentration, and volume of the ^{177}Lu-PSMA-617 (table 2). The stability persisted even considering the worst-case scenario, that is, greater labeling activity (37 GBq) and the doses dispensed in the smallest volume (2.5 mL) (figure 3).

Table 2. Labeling conditions, quality control results of ^{177}Lu-PSMA-617 produced batches (stored under freezing), and acceptance criteria.

Labeling Activity (GBq)	^{177}Lu Supplier	Radiochemical Purity (%) in TLC			Sterility Assay	Pyrogen Assay	Radionuclidic purity (%)	pH
		Immediately	24 hours	48 hours				
9.25	NTP	98.8	98.7	98.4	Pass	Pass	Pass	4.5
18.5	NTP	99.4	98.9	98.6	Pass	Pass	Pass	4.5
18.5	NTP	99.0	98.3	97.1	Pass	Pass	Pass	4.5
18.5	NTP	98.9	98.3	98.8	Pass	Pass	Pass	4.5
18.5	NTP	99.5	98.9	98.3	Pass	Pass	Pass	4.5
7.4	INM	99.6	99.5	99.8	Pass	Pass	Pass	4.5
18.5	INM	99.5	NR	NR	Pass	Pass	Pass	4.5
25.9	INM	99.5	99.5	99.4	Pass	Pass	Pass	4.5
37	INM	99.2	99.6	99.6	Pass	Pass	Pass	4.5
37	INM	99.7	99.5	99.4	Pass	Pass	Pass	4.5

Acceptance criteria (European Pharmacopoeia, 10 ed.), Radiochemical purity: ≥95%; sterility: absence of microbiological growth in 14 days; Pyrogen: ≤ 175EU/V; Radionuclidic Purity: ≥99.9% of ^{177}Lu, ^{175}Yb≤0.1,^{177m}Lu ≤0.07%; pH: 4.0 – 5.0. NR = unrealized

After the radiolabeling procedure, DTPA solution was introduced as a chelating agent for free lutetium-177, to complex with any $^{177}Lu^{+3}$ not labeled to the PSMA-617 or released from them. This chelation does not reduce the percentage of radiochemical impurity but contributes to the faster renal elimination of the lutetium-177 and minimizes the impact on the bone marrow [7]. The DTPA showed an ability to chelate 90% of free lutetium in the final product after 24 hours at room temperature, by an experiment that simulated an eventual release of 5% of lutetium-177 used in the radiolabeling procedure. In fact, it has been previously described that DTPA, forms a complex with lutetium-177 at room temperature and acidic pH [8]. In these circumstances, the DTPA can chelate the free lutetium released from the radiopharmaceutical by the effect of radiolysis, which may occur to the dose in the liquid state in two different conditions/circumstances: when it takes too long to freeze before the transportation, or when it takes too long to administrate after defrosting.

Another reason for DTPA addition is the difficulty in determining of RCP in HPLC, because the $^{177}LuCl_3$ can bind at the origin of the column resulting in false identification of radiochemical purity in this chromatographic system. Additionally, this may occur in ITLC (instant thin layer chromatography) control [9].

The retention times of the ^{177}Lu-DTPA and the $^{177}LuCl_3$, 3.8 and 3.9 min respectively, were too close, making it difficult to distinguish them by HPLC. In order to distinguish these compounds, two different chromatographic systems (0.1 M citrate buffer pH 5.0 and 1.0 M ammonium acetate pH 8.5: methanol 1:1 v/v) were combined for identification and quantification of these impurities by TLC-SG. In the stability studies, the ^{177}Lu-DTPA complex was not detected, probably due to freezing storage.

Saline isotonic solution was added for dilution of the radiopharmaceutical and the final pH for all batches was 4.5, showing that the volume and the molarity (0.52 M) of the ascorbate buffer not only made it possible to maintain the appropriated condition for radiolabeling after the introduction of the acidic lutetium-177 chloride solution, but also made it possible to obtain the final product within the pH pharmacopoeia criteria.

A different stability study reported that the radiolysis of the ^{177}Lu-PSMA-617 at 4 GBq was mitigated at room temperature for 24 hours, despite the fact that the radiochemical concentration was lower than the one used in this present study (0.23 GBq/mL and 40 MBq/µg). The authors showed that the ascorbic acid at a concentration of 1.08 mg/mL maintained the radiochemical purity over than 95% [10]. The higher ascorbic acid concentration (5 mg/mL) used in this study was not able to prevent radiolysis at radiochemical concentration of 0.74 GBq/mL, though. However, in the stability study in this work for doses of 7.4 GBq (74 MBq/µg) obtained from a 37 GBq batch, the combination of ascorbic buffer, property dilution (radioactive concentration ≤ 3.7 GBq/mL), and freezing resulted in appropriate stability over a 48-hour period.

The final volume of the therapeutic doses is a critical factor to be considered when planning the production of industrial batches. Smaller volumes facilitate the sterilizing filtration process and dose fractionation, especially when considering semi-automated processes. In this work, the final volume of the batches did not exceed 10 mL, and this facilitated the sterilizing filtration process, and reduced the risks of aseptic packaging of the radiopharmaceutical. Moreover, a higher concentration of ascorbate buffer was needed to mitigate the radiolysis.

The use of higher radioactive concentrations increases the risk of radiolysis of the preparation. In this case, reducing the temperature by freezing the doses was used to compensate for the radioactive concentration employed, because it minimizes the transit of the free radicals produced in the water by the β-particles emitted by ^{177}Lu [11].

The stability of the ^{177}Lu-PSMA-617, even at the concentration of 3.7 GBq/mL, was undoubtedly related to the use of an antioxidant buffer (sodium ascorbate and ascorbic acid), the dilution after the radiolabeling procedure, and the freezing process.

The results of this study encourage the scale up of the production of batches up to 111 GBq, in the same way as the ^{177}Lu-DOTATATE lots produced at IPEN/Brazil. The results could also be used as starting point for stability studies with ^{177}Lu-PSMA-I&T, which will be used in a future study and clinical trials for prostate cancer treatment in Brazil.

Also, the radiolysis effect was mitigated by the antioxidant properties of the ascorbate buffer.

4. CONCLUSION

This work described the preparation of industrial batches of ^{177}Lu-PSMA-617, with activity up to 37 GBq, and the fractionation to therapeutic doses of 7.4 GBq, calibrated for 48 hours. The stability found to therapeutic doses, based on the combination of factors such as final radioactive concentration, presence of stabilizers and low temperature ensured transport to regions far from the production site.

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