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Radiochemical Stability Study of Therapeutic doses of Lu-177-PSMA-I&T.

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1. Introduction

Prostate cancer mainly affects men aged between 45-60 years, the third neoplasm with the most cases and the disease with the highest diagnosis rate among men in the world [1,2].
Prostate Specific Membrane Antigen (PSMA), is a glycoprotein type II and there is a consensus among authors describing a strong correlation between PSMA expression and the malignancy of prostate cancer [3,4,5]. PSMA-related radiopharmaceuticals were extensively studied from the point of view of radiolabeling and clinical application, using radionuclides for diagnostic and therapy. In therapeutic application, the labeling of PSMA related analogs with lutetium-177 (^{177}Lu) stands out, with the characteristic of stability after labeling being a challenge to the transport and clinical application of these radiopharmaceuticals, due to the degradation caused by radiolysis [4,6].
Several agents promote the stability of therapeutic radiopharmaceuticals, such as dilution, freezing and the use of stabilizing agents such as diethylenetriaminepentaacetic acid (DTPA) gentisic acid, ascorbic acid and ethanol. The stabilizing agents, however, are part of the final formulation of the radiopharmaceutical, making relevant toxicological aspects [6,7]. The stability of the radiopharmaceutical for therapy is also directly related to the specific activity of the preparation.
This present work aims to evaluate the radiochemical yield and stability of ^{177}Lu-PSMA-I&T therapeutic doses, prepared with stabilizer buffer in different peptide to lutetium molar ratios.

2. Methodology

2.1 Materials and Methods

The radiolabeling of PSMA-I&T (CMR-Russia) with lutetium trichloride ($^{177}LuCl_3$) was based and adapted on the methodology described by Villas Boas [8]. The preparation of therapeutic doses was determined with 9.25 GBq (250 mCi) of lutetium-177 and 313 to 496 µg of PSMA-I&T peptide, aiming to compare different peptide to lutetium molar ratios. The sodium ascorbate buffer pH 4.7 was chosen for labeling due to its protective properties against the effects of radiolysis. DTPA solution was added after labeling procedure.

2.2 Quality Control

The percentage of radiochemical purity (%RP) was determined by High-performance liquid chromatography composed by automatic injector unit (SIL-AC20), pump (LC-20AT), DGU-20 SR degasser, UV/VIS detector (SPD-20ª), CBM-20ª communicator module (Shimadzu, Japan) and Laura 4.0 software for controlling the HPLC System (Lablogic, England). A gamma radiation detector was attached to this unit (Flow-RAM Radio HPLC Detector, Lablogic, England).

According to the method described by Villas Boas [8], analysis was conducted using a C-18 column (Waters model Xterra RP 18 column 5 µm, 4.6 x 150 mm, 24ºC), with injection volume of 100 µL, and gradient of 0-8.59 min 24% B; 9-18 min 60% B; flow 0.6 mL/min, A= Water/0.1% trifluoroacetic acid, B= acetonitrile/0.1% trifluoroacetic acid.
Radiochemical Purity by Thin Layer Chromatography was determined using silica gel 60 (TLC-SG) coated aluminum strips (stationary phase) and 0.1M sodium citrate buffer, pH 5.0 (mobile phase), and the radioactivity was detected with Gamma Counter (Packard, USA). The obtained results were confronted with the internal acceptance criterion (%RP ≥ 97%) still under evaluation.
The stability of the therapeutic doses kept frozen (-25ºC) was assessed by HPLC and CCD analysis, performed immediately (N = 3), 24 hours (N= 3) and 48 hours (N=2) after labeling for peptide to lutetium rate of 3.0 to 3.4 and N=3 for peptide to lutetium rate of 3.6 to 4.0.

3. Results and Discussion

The stability of ^{177}Lu- PSMA-I&T was studied in therapeutic doses, prepared with different peptide to lutetium molar ratios, to evaluate the impact of the final specific activity of the radiopharmaceutical on stability.
In Figure 1, the %RP of therapeutic dose stability study using TLC (A) and HPLC (B) are representative mean and standard deviation of molar ratio 3.0 to 3.4. For ANOVA test (p-value <0.05) the difference was not significant, considering time after labeling (P=0.2999), but, for HPLC analysis, the results do not meet the internal acceptance criteria for %RP (≥97%) of ^{177}Lu- PSMA-I&T, after 24 hours and 48 hours stored under freezing.

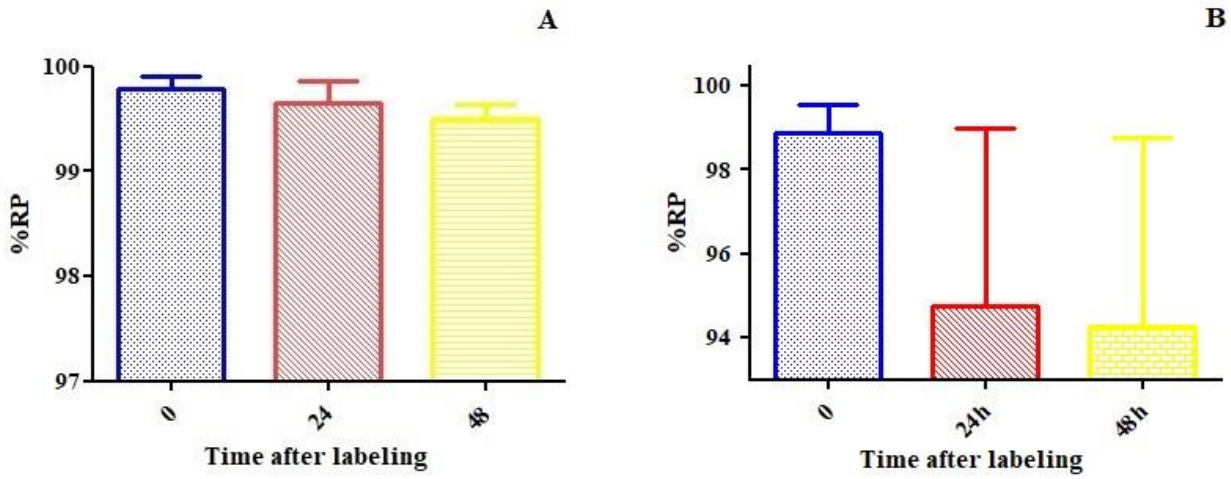

Figure 1: %RP of therapeutic dose of ^{177}Lu-PSMA-I&T prepared with molar ratio 3.0 to 3.4 and stored under freezing: (A) TLC analysis; (B) HPLC analysis.

Figure 2 presents the %RP of therapeutic dose stability study using TLC (A) and HPLC (B) and is representative mean and standard deviation of molar ratio 3.6 to 4.0. ANOVA test (p-value <0.05) does not demonstrate a significant difference between times (P=0.5011). The HPLC results demonstrate that the preparation remained stable, meeting the internal acceptance criteria (≥ 97% RP) up to 48 hours after preparation, in the proposed storage conditions.

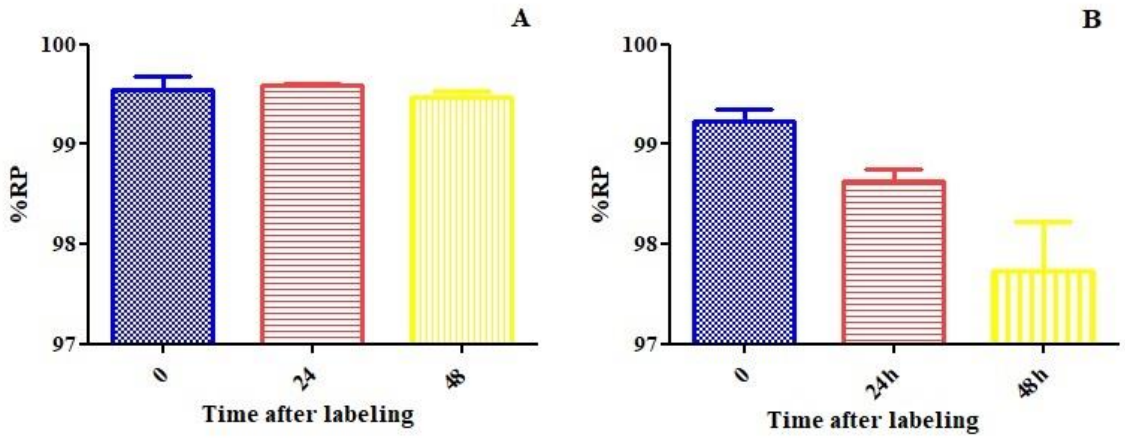

Figure 2: %RP of therapeutic dose of ^{177}Lu-PSMA-I&T prepared with molar ratio 3.6 to 4.0 and stored under freezing: (A) TLC analysis; (B) HPLC analysis.

Figure 3 shows typical HPLC chromatograms for different molar ratios used in the preparation of ^{177}Lu-PSMA-I&T, 24 hours after labeling demonstrating additional peaks and decreased %RP.

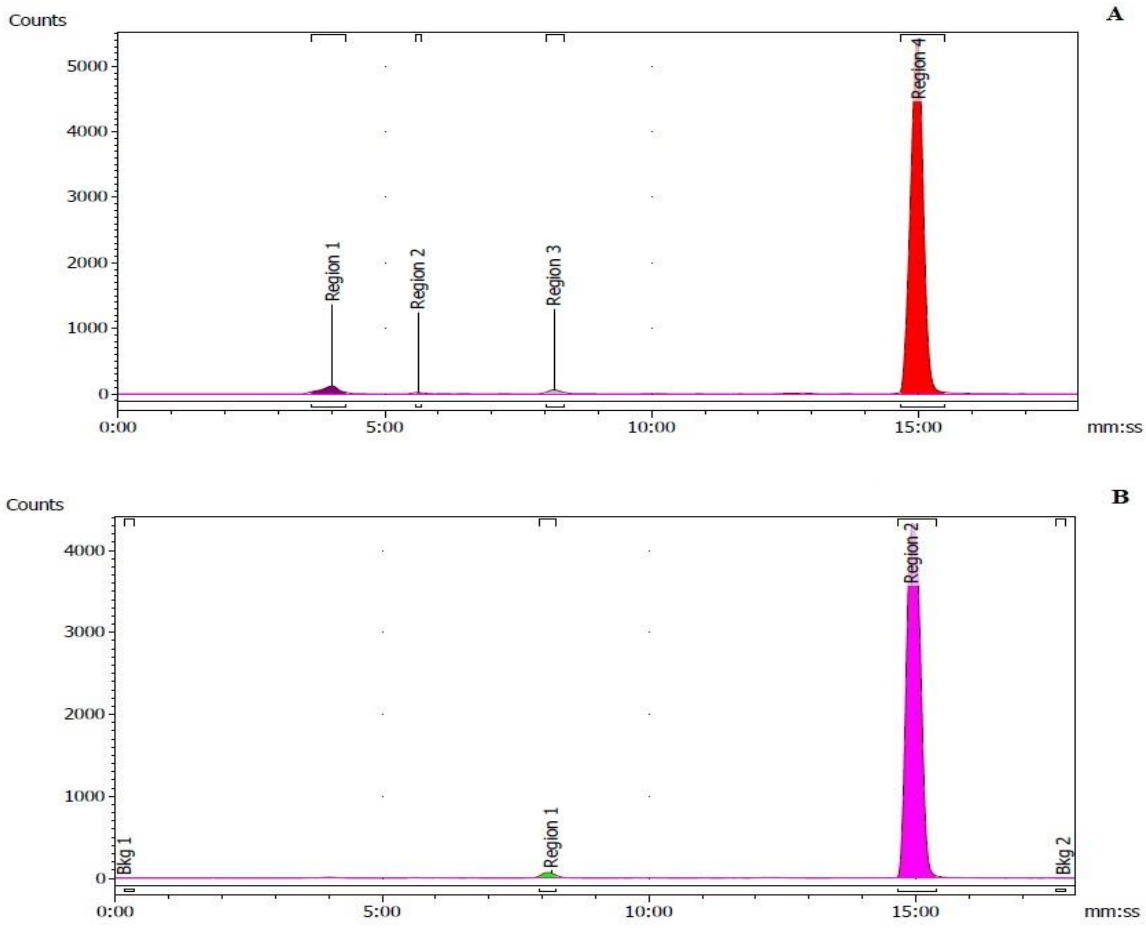

Figure 3: HPLC chromatograms of therapeutic doses of ^{177}Lu-PSMA-I&T: (A) molar ratio 3.0 to 3.4 (%RP of main peak in red = 95.7 %) and (B) molar ratio 3.6 to 4.0 (%RP of main peak in pink = 98.9 %) stored under freezing for 24 hours.

4. Conclusions

This preliminary study made it possible to determine the peptide to lutetium molar ratio range that provides greater stability to the radiopharmaceutical over the proposed storage period and conditions. Additional studies will be carried out to assess the impact on production scheduling.

Acknowledgements

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