J of Nuclear Medicine Technology, first published online June 14, 2022 as doi:10.2967/jnmt.121.262423

Title:
Stability matters: Radiochemical stability of therapeutic radiopharmaceutical of 177Lu-PSMA-I&T

Running title:
Radiochemical stability of PSMA-I&T

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Word count
1810 words

Financial support disclosure

There was no financial support received for the current study from any organisation.

Abstract

Labelling radiopharmaceuticals and testing the quality of the labelled product before injecting it into patients are standard operating procedures in the Nuclear Medicine department. There is a different shelf life for each labelled product, which determines how long a product can maintain in-vitro stability before it needs to be discarded. Lutetium-177 (177Lu) is a radioactive isotope that is increasingly being accepted into the treatment paradigm for palliation of advanced-stage tumours, including metastatic castration-resistant prostate cancer (mCRPC) and neuroendocrine tumours (NET).

In our institution, synthesis of 177Lu with prostate-specific membrane antigen imaging and therapy (PSMA-I&T) for palliation of mCRPC is performed on Eckert & Ziegler Eurotope's Modular-Lab Pharm Tracer® automated synthesis system. Sterile GMP-certified no-carrier-added 177Lu is supplied by Australia's Nuclear Science and Technology Organization (ANSTO). Following each synthesis, the final product quality is evaluated by High-performance liquid chromatography (HPLC) and instant thin-layer chromatography (ITLC) at three different time points: 0 hours, 24 hours, and 48 hours. Between February 2020 to October 2020, the quality of 35 batches of 177Lu-PSMA-I & T was evaluated. The average radiochemical purity of ITLC-SG was found to be greater than 99 percent (99.70±05%), and HPLC was greater than 98 percent (98.60±0.05%).

Our findings demonstrate that automated synthesis of 177Lu-PSMA-I & T with Eckert & Ziegler Eurotope's Modular-Lab Pharm Tracer® can remain stable for 48 hours post labelling.

INTRODUCTION

Patients with metastatic castration-resistant prostate cancer (mCRPC) have disease progression despite using maximum androgen blockade, as evidenced by a low testosterone level[*1*]. It is therefore an advanced, and usually end stage form of prostate cancer. Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein with enzymatic properties that is anchored in the cell membrane of prostate epithelial cells and is overexpressed by prostate cancer cells[*2*]. As a result, PSMA can be used as a biomarker for prostate cancer. For peptide receptor radionuclide therapy, PSMA peptides can be radiolabelled with the beta emitter radioisotopes Yttrium-90 (90Y) and 177Lu (PRRT). PSMA-I&T, PSMA-617, and J591 are three analogues frequently used in therapy[*2*]. Due to its favourable physical characteristics [Half Life (t1/2)=6.73 d, Mean Energy of -particle (Emax) =0.497 MeV, and Mean Energy of particle (Emax) =0.497 MeV], 177Lu has been identified as one of the most promising radionuclides for therapeutic applications[*3*]. The beta-particle emitted by 177Lu has a short path length of 1.5mm, allowing it to deliver effective tumour radiation while causing minimal damage to surrounding normal tissues. The use of the two primary-gamma energies of 113 and 208 keV, respectively, allows for the use of SPECT imaging after treatment[*4*]. The aim of this work was to evaluate the stability of Lu-177-PSMA-I&T using the HPLC and TLC methods. This evaluation will help understand the shelf life of the labelled product, which might be helpful for logistic purposes.

MATERIALS AND METHODS

The labelling of Lutetium trichloride ($177LuCl_3$) and peptide (PSMA-I&T;40 mg Ascorbic acid and 10 mg Sodium hydroxide) were prepared with a fully automated radiopharmaceutical synthesis device using Modular-Lab Pharm Tracer by Eckert & Ziegler. PSMA-I&T was labelled with 177Lu using good manufacturing practice (GMP)-grade disposable cassettes and reagent kits supplied by ABX Advanced Biochemical Compounds (See fig.1). Labelling of 177Lu-PSMA-I&T was done as per the ABX and Eickert & Zeigler synthesis instructions with the help of a pressure-based cassette. The non-carrier added (NCA) $177Lucl_3$ was supplied by ANSTO, Australia and ITG (Isotope Technologies Garching GmbH, Germany). Radiations quantity was procured based on the number of patients treated per cycle. The required dose for one patient was usually 7 GBq based on the estimated synthesis yield of 80%. ABX supplied the accessory chemical, including Sodium hydroxide-Ascorbic acid and 50 ml Sodium chloride

(saline) along with 0.55mico mole filters, long needles and vent needles. PSMA-I&T was supplied by Huwai chem in a 1miligram (mg) vial, which was fractionated into 200 micrograms (µg) and were stored in the freezer. The required amount of PSMA-I&T (200 µg/5GBq) was reconstituted with 1.5 ml Sodium ascorbate (0.57 M) to adjust the pH to 4.5±0.1. Labelling is performed with the help of a computer-based automated system that executes with the help of software. All production cassettes were supplied by vendors and were made for single use only.

Shelf life of prepared 177Lu-PSMA I&T was established based on the evaluation of radiochemical purity by High-Performance Liquid Chromatography (HPLC) and Instant Thin Layer Chromatography-Silica Gel (ITLC-SG) [*5*].

HPLC was used for radiochemical analyses and purification of the 177Lu-labelled PSMA-I&T conjugates. A dual pump HPLC unit with a C18 reverse-phase column (25 cm × 0.46 cm) purified the labelled conjugates. Mixtures of 1% trifluoroacetic acid, Ultrapure water (solvent A), and 0.1% trifluoroacetic acid and acetonitrile (solvent B) are used as the mobile phase.[*6*] The following gradient elution technique is adopted for the separation,0–3 min, A: 100% B:0%;3-10min, A:50%,B:50%;10-15min, A: 0%, B: 100%.HPLC analysis showed that the fast eluting compound was hydrophilic 177Lu cation (1.0 min), while 177Lu-PSMA-I&T with high molecular weight was eluted after 8 min, as seen in the chromatogram diagram. The typical retention time of radiolabeled PSMA under the above conditions is approximately 500 s (8-10min).

ITLC-SG method was applied to check the radiochemical purity of the radiolabelled complex. The principle of the ITLC-SG analytical method is that a mobile phase moves along a stationary phase due to capillary forces. Therefore, depending on the distribution of components between the stationary and the mobile phase, a radioactive sample spotted in the adsorbent will migrate with different velocities, and thus impurities are separated. Using a 10 cm long Whatman 3MM chromatography paper's stationary phase, the study was carried out. For these studies, five µL of the test solution was spotted at 1.5 cm from the lower end of the paper strips, developed in 10% ammonium acetate in methanol as mobile phases (30:70 vol./vol.) Following each synthesis, the given amount of radiolabelled complex (approximately 10 MBq) was kept at room temperature for 48 hours while being checked by HPLC & ITLC-SG at specified time intervals: 0 hours, 24 hours, and 48 hours after preparation (See table 1 and 2).

RESULTS

Thirty-five batches of ^{177}Lu-labelled PSMA-I&T were completed between February 2020 to October 2020. The average radiochemical purity of ITLC-SG was greater than 99 per cent (99.70.±05 per cent), and HPLC was greater than 98 percent (98.60±0.05%) at room temperature at both 24 and 48 hours post-synthesis which was consistent with the various concentration of the 177Lu-PSMA-I&T (see supplemental table 1; figure 2,3 and 4)

DISCUSSION

Most of the radiopharmaceuticals required to be a radiochemical purity (RCP) of >95%. It is always good to know how long a product can stay stable. It enables it to be transportable in distant places, especially in the current Covid-19 situation where travelling is quite a complex procedure for the patients. The current study found that labelled Lu-177-PSMA-I&T stays stable for up to 48 hours, which justifies the manufactured product and the influential role of applied quenchers. Both HPLC and ITLC-SG has been found to agree with final product stability. In our formulation, we applied sodium ascorbate as a quencher with a concentration of 2 mg/ml. It is to be noted here that all the results have been evaluated based on the suggested methods by Eckert & Ziegler Eurotope's Modular-Lab Pharm Tracer ©.

CONCLUSION

Our study demonstrates that automated synthesis of Lu177-PSMA I&T with the Eckert & Ziegler Eurotope's Modular-Lab Pharm Tracer® can remain stable for 48 hrs. This suggests it is feasible for pre-labelled177Lu-PSMA-I&T to be supplied from a source location to distant satellite clinics, potentially improving access to PSMA directed radioligand therapy for palliation treatment mCRPC**.**

Disclaimer

No potential conflicts of interest relevant to this article exist.

Key Points

Question

Is the Lu-177-PSMA-I&T stable enough to allow it to be transported?

Pertinent Findings

Lu-177 labelled with PSMA-I&T was found to be stable for up to 48 hours in our study. This finding is encouraging because it suggests that labelled products can be transported from one location to another.

Implications for Patient Care

The current finding is encouraging because it suggests that the Lu-177-PSMA-I&T has a long enough shelf life. In addition, it opens the door to the possibility of patients receiving treatment without having to travel away from their home locations.

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Figures

Figure 1: Modular-Lab Pharm cassettes supplied by Eckert and Zeigler

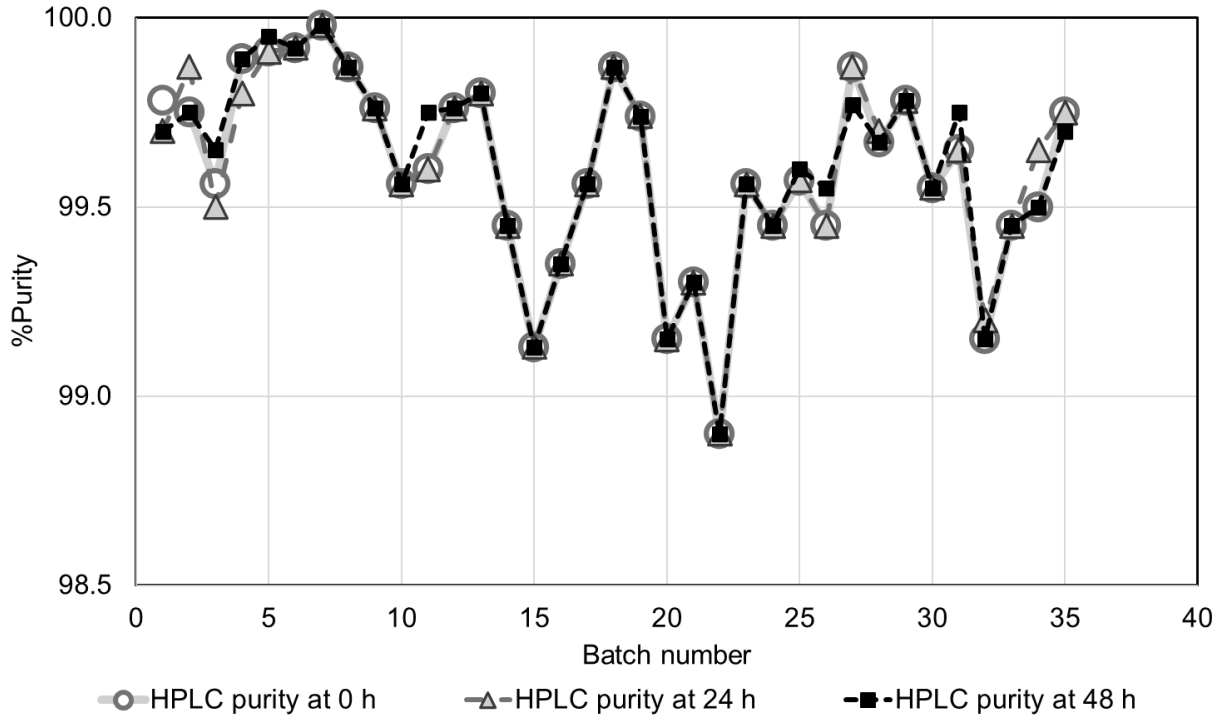


Figure 2: A line graph showing HPLC-SG QC result comaprison at 0,24 and 48 hrs

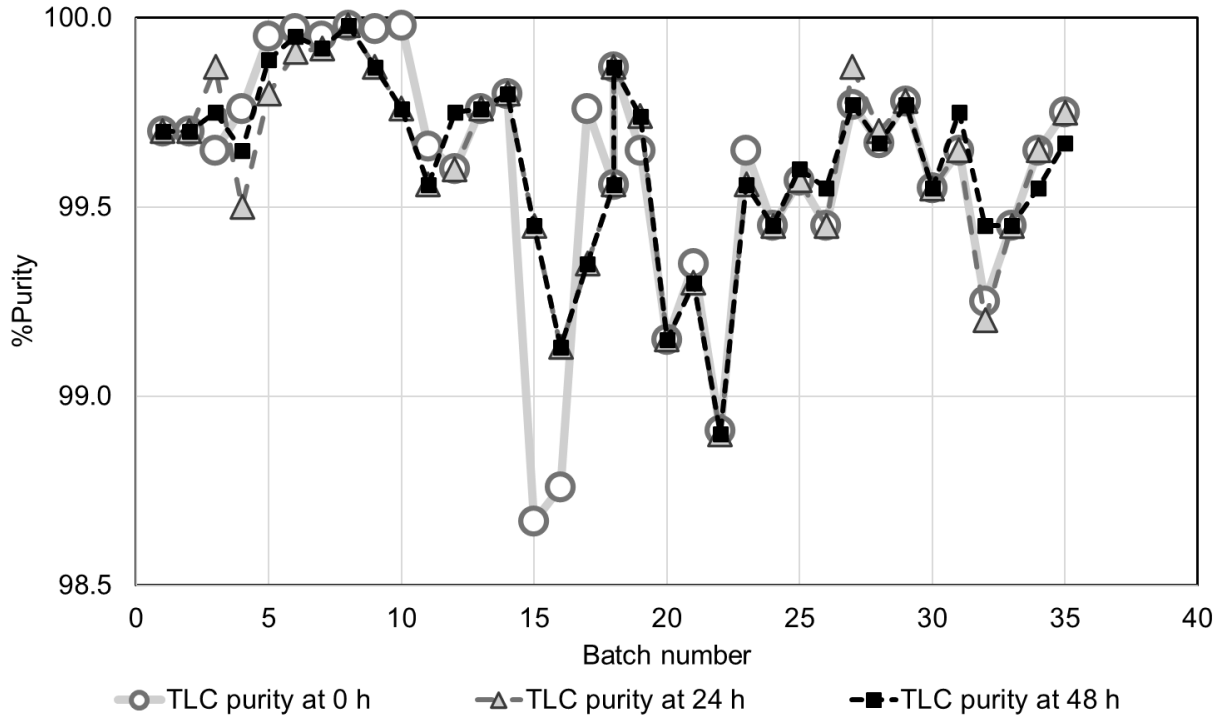


Figure 3: A line graph showing ITLC-SG QC result comaprison at 0,24 and 48 hrs

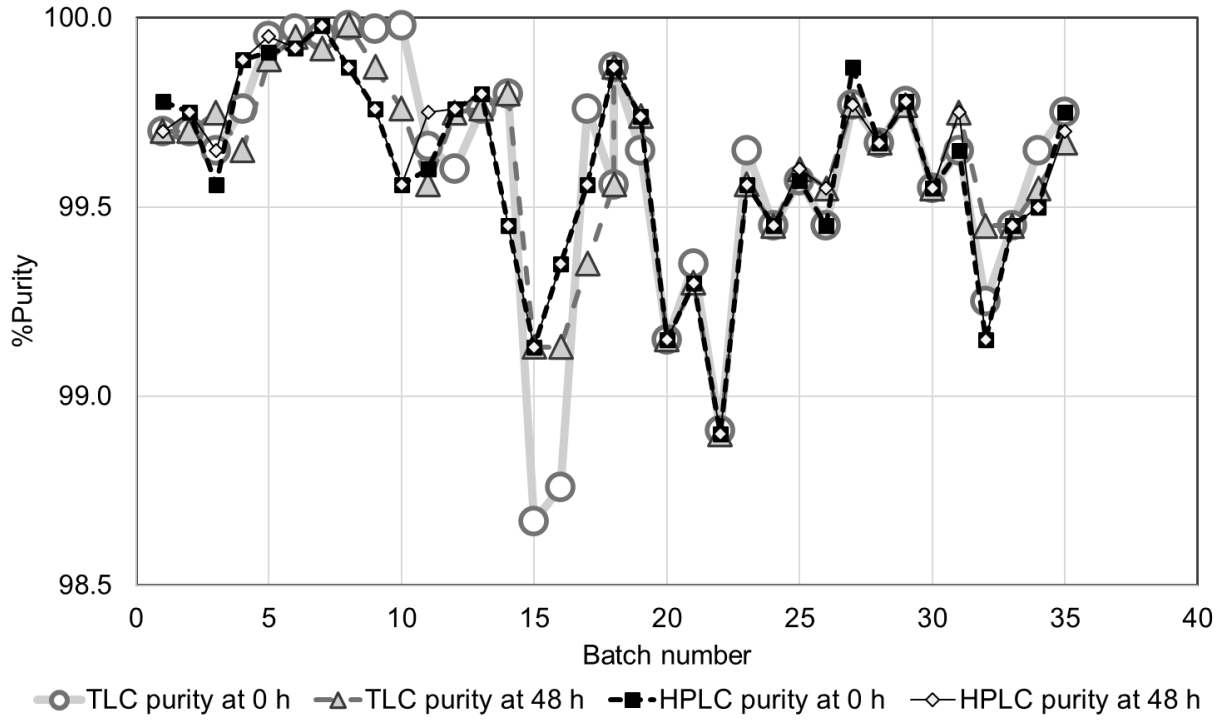


Figure 4: A line graph showing result of HPLC and ITLC-SG at 0 and 48 hours

Batch Number	HPLC purity result at 0 hour	HPLC purity result at 24 hours	HPLC purity result at 48 hours
1	99.78	99.7	99.7
2	99.75	99.87	99.75
3	99.56	99.5	99.65
4	99.89	99.8	99.89
5	99.91	99.91	99.95
6	99.92	99.92	99.92
7	99.98	99.98	99.98
8	99.87	99.87	99.87
9	99.76	99.76	99.76
10	99.56	99.56	99.56
11	99.6	99.6	99.75
12	99.76	99.76	99.76
13	99.8	99.8	99.8
14	99.45	99.45	99.45
15	99.13	99.13	99.13
16	99.35	99.35	99.35
17	99.56	99.56	99.56
18	99.87	99.87	99.87
19	99.74	99.74	99.74
20	99.15	99.15	99.15
21	99.3	99.3	99.3
22	98.9	98.9	98.9
23	99.56	99.56	99.56
24	99.45	99.45	99.45
25	99.57	99.57	99.6
26	99.45	99.45	99.55
27	99.87	99.87	99.77
28	99.67	99.7	99.67
29	99.78	99.78	99.78
30	99.55	99.55	99.55
31	99.65	99.65	99.75
32	99.15	99.2	99.15
33	99.45	99.45	99.45
34	99.5	99.65	99.5
35	99.75	99.75	99.7

Table 1: HPLC Results at various time period

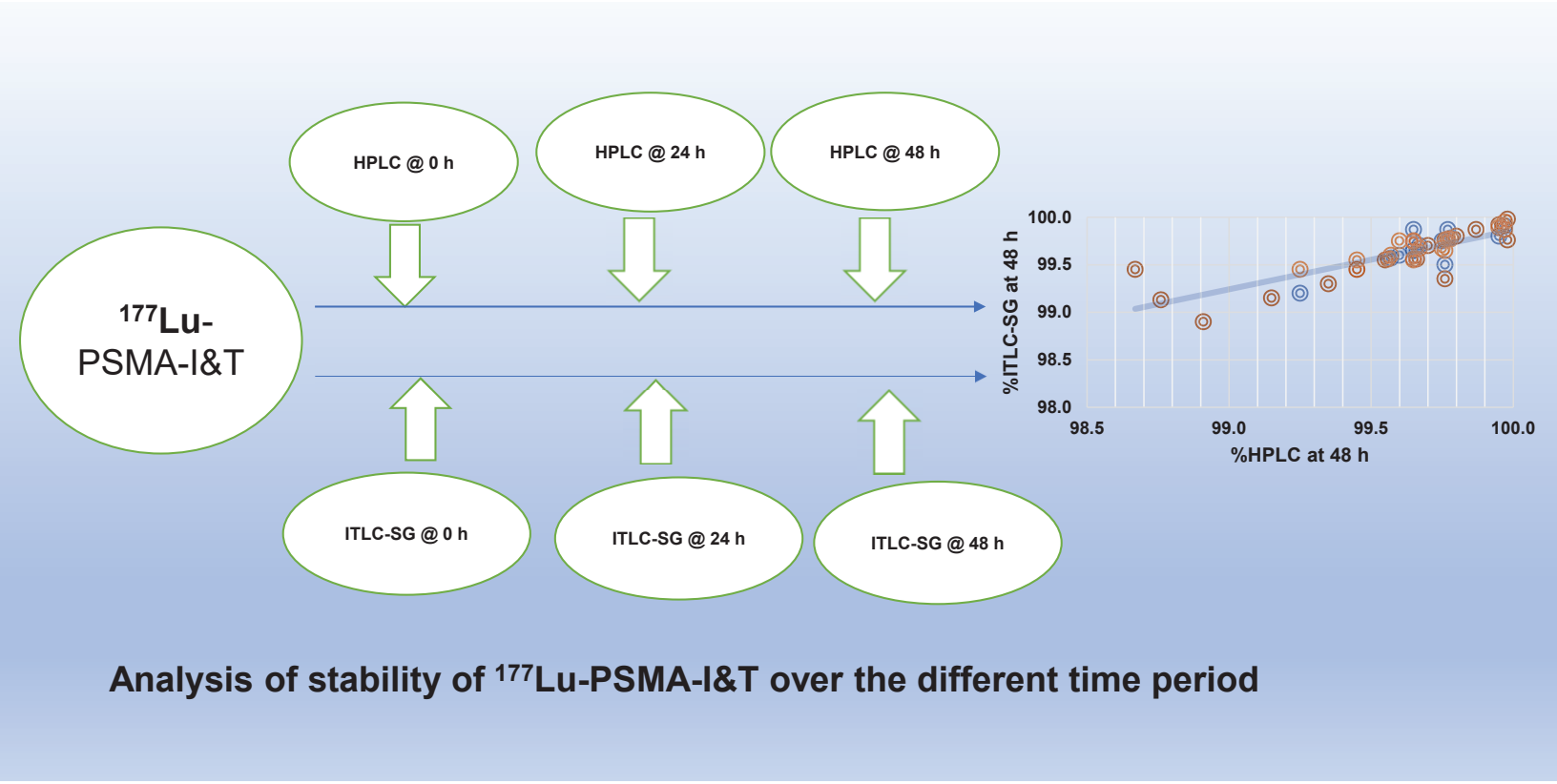
Batch Number	TLC purity result at 0 hour	TLLC purity result at 24 hours	TLC purity result at 48 hours
1	99.70	99.7	99.7
2	99.65	99.87	99.75
3	99.76	99.5	99.65
4	99.95	99.8	99.89
5	99.97	99.91	99.95
6	99.95	99.92	99.92
7	99.98	99.98	99.98
8	99.97	99.87	99.87
9	99.98	99.76	99.76
10	99.66	99.56	99.56
11	99.60	99.6	99.75
12	99.76	99.76	99.76
13	99.80	99.8	99.8
14	98.67	99.45	99.45
15	98.76	99.13	99.13
16	99.76	99.35	99.35
17	99.56	99.56	99.56
18	99.87	99.87	99.87
19	99.65	99.74	99.74
20	99.15	99.15	99.15
21	99.35	99.3	99.3
22	98.91	98.9	98.9
23	99.65	99.56	99.56
24	99.45	99.45	99.45
25	99.57	99.57	99.6
26	99.45	99.45	99.55
27	99.77	99.87	99.77
28	99.67	99.7	99.67
29	99.78	99.78	99.77
30	99.55	99.55	99.55
31	99.65	99.65	99.75
32	99.25	99.2	99.45
33	99.45	99.45	99.45
34	99.65	99.65	99.55
35	99.75	99.75	99.67

Table 2: ITLC-SG Results at various time period

Batch Number	Initial activity of Lu-177-PSMA-I&T	Lu-177-PSMA-I&T activity at 48 hours
1	25.6 GBq in 25 ml	20.7 GBq in 24.5 ml
2	28.6 GBq in 25 ml	23.12 GBq in 24.6 ml
3	14.6 GBq in 20 ml	11.72 GBq in 19.5 ml
4	14.1 GBq in 20 ml	11.32 GBq in 19.6 ml
5	22.3 GBq in 25 ml	17.9 GBq in 24.6 ml
6	31.2 GBq in 25 ml	25.12 GBq in 24.5 ml
7	28.5 GBq in 25 ml	23.0 GBq in 24.4 ml
8	8.7 GBq in 15 ml	6.99 GBq in 14.6 ml
9	11.28 GBq in 15 ml	8.91 GBq in 14.5 ml
10	32.3 GBq in 25 ml	25.8 GBq in 24.7 ml
11	31.2 GBq in 25 ml	25.1 GBq in 24.6 ml
12	13.5 GBq in 15 ml	10.25 GBq in 14.6 ml
13	26.3 GBq in 20 ml	21.10 GBq in 19.6 ml
14	14.6 GBq in 20 ml	11.25 GBq in 19.5 ml
15	14.1 GBq in 20 ml	11.22 GBq in 19.6 ml
16	22.3 GBq in 25 ml	17.91 GBq in 24.6 ml
17	31.2 GBq in 25 ml	24.85 GBq in 24.6 ml
18	28.5 GBq in 25 ml	23.05 GBq in 24.6 ml
19	8.7 GBq in 15 ml	6.99 GBq in 14.6 ml
20	11.28 GBq in 15 ml	9.05 GBq in 14.6 ml
21	14.6 GBq in 20 ml	11.65 GBq in 19.6 ml
22	14.7 GBq in 20 ml	11.25 GBq in 19. 6 ml
23	21.3 GBq in 25 ml	16.95 GBq in 24.6 ml
24	31.5 GBq in 25 ml	25.23 GBq in 24.6 ml
25	28.5 GBq in 25 ml	23.0 GBq in 24.6 ml
26	8.7 GBq in 10 ml	6.99 GBq in 9.7 ml
27	17.8 GBq in 15 ml	14.23 GBq in 14.7 ml
28	16.6 GBq in 20 ml	13.20 GBq in 19.6 ml
29	16.1 GBq in 20 ml	12.93 GBq in 19.6 ml
30	27.3 GBq in 25 ml	21.90 GBq in 24.6 ml
31	32.2 GBq in 25 ml	25.91 GBq in 24.6 ml
32	29.5 GBq in 25 ml	23.45 GBq in 24.6 ml
33	8.7 GBq in 15 ml	6.95 GBq in 14.7 ml
34	13.5 GBq in 15 ml	10.65 GBq in 14.6 ml
35	12.4 GBq in 15 ml	9.87 GBq in 14.6 ml

Supplemental Table 1: Initial and 48 hours Radioactivity Concentration of Lu-177-PSMA-I&T

Graphical Abstract



Analysis of stability of ^{177}Lu-PSMA-I&T over the different time period