PREPARATION AND EVALUATION OF PHARMACEUTICAL-GRADE DTPA KIT RADIOLABEL WITH ^{99m}TC

Muhammad Zaki Abdullah[a], Ahmad Rashidi Mohamed Tahir [b]*, Wan Hamirul Bahrin[c]

Article History: **Received**: 28.07.2022 **Revised**: 27.08.2022 **Accepted**: 27.09.2022

Abstract: Nuclear medicine is one of the medical imaging modalities. It utilizes the use of radiopharmaceutical for the purpose of diagnostic or therapeutic for the patient. The term of radiopharmaceutical consists of two main components which are radionuclide and pharmaceutical. Radionuclide act as tracer which can be detected by the special camera while pharmaceutical often act as ligand to bring the radionuclide to the targeted organ. In this study, Diethylenetriaminepentaacetic acid (DTPA) which is a pharmaceutical that targeting kidney was being produced in the kit form by using freeze drying method to increase its stability and shorten its preparation time before radiolabelling. The DTPA kit is radiolabelled with the ^{99m}Tc ($t_{1/2}$ = 6 hours) to form ^{99m}Tc-DTPA complex before injecting to the patient. Since this radiopharmaceutical is intended for the patient use, a thorough quality control must be done to ensure its safety and efficacy. Certificate of Analysis (CoA) of DTPA kit that was obtained from Quality Control Unit of Medical Technology Division, Malaysian Nuclear Agency proves the safety and efficacy of the kit to be legally supplied for the patient use.

Keywords: DTPA kit, Technetium-99m, Freeze drying, renal scintigraphy, pharmaceutical-grade

[a]. Department of Pharmaceutical Technology and Industry Faculty of Pharmacy, University of Cyberjaya, 63000 Cyberjaya, Selangor, Malaysia.
[b]. Department of Pharmaceutical Technology and Industry Faculty of Pharmacy, University of Cyberjaya, 63000 Cyberjaya, Selangor, Malaysia.
[c]. Radiopharmaceutical Technology Group, Medical Technology Division, Malaysia Nuclear Agency, Malaysia.

***Corresponding Author**

E-mail: rashidi@cyberjaya.edu.my

DOI: 10.31838/ecb/2022.11.07.015

INTRODUCTION

Nuclear medicine study involves administration of labelled compound with gammaray-emitting or positron-emitting radionuclide in the body to get diagnostic information of the disease commonly by injection into the patient's body. The compound that is radiolabelled with the radionuclide is called radiopharmaceutical. The advantage of using nuclear medicine compared to other medical imaging modalities is it can provide exquisitely sensitive measure of biologic process in the body other than it also can provide outstanding anatomic images like other medical imaging modalities [1]. Renal scintigraphy is one of the nuclear medicine techniques that uses radionuclide to evaluate the renal function. The major advantage of renal scintigraphy using nuclear medicine is it can evaluate kidney functional status that cannot be obtained from other renal test such as blood urea nitrogen (BUN) and biochemical assessment of serum creatinine. Data that obtained from renal scintigraphy can be used along with other data obtained through other imaging technique can assist the clinician in diagnosis and management of various renal disorder [2]. One of the most frequently used radiopharmaceuticals for renal scintigraphy is ^{99m}Tc-DTPA. DTPA is a ligand that form complex with the radionuclide such as ^{99m}Tc through the process of radiolabelling. It is possible to be produced in DTPA kit which is one of the frequently used instant kits in the nuclear medicine [3]. The advantage of producing DTPA in kit form is it facilitate robust and high-yielding ^{99m}Tc labeling reactions using simple and typically one-step procedures [4]. Strict quality control measure is important for the radiolabelled compound since it is intended to use for human administration. the quality controls of the radiolabelled compound are done to ensure its efficacy and safety when injecting to human. The quality control tests fall into two categories: physicochemical test such as radiochemical purity and biological test such as sterility and apyrogenicity [5].

METHODS

Materials

^{99m}Tc eluted from ^{99}Mo/^{99m}Tc Generator (Curium, France), Water for Injection (WFI),nitrogen gas, stannous chloride, 10M HCl, Calcium Trisodium DTPA, 2M NaOH, 1M HCl, normal saline, acetone, amoebocyte lysate, Fluid thioglycollate medium, Soya- bean Casein digest medium, Human Serum Albumin (HSA), isoflurane gas, male Sprague Dawley rats.

Equipments

Oven, autoclave, vortex, micropipette, pH meter, 25 ml volumetric flask, 0.22 µm filter (Sartorius Stedim Biotech, Germany), Freeze Dryer (Virtis Genesis, USA), lead shield, Dose Calibrator (Biodex Medical System, New York), Bioscan® AR-2000 Radio-TLC Imaging Scanner (Eckert & Ziegler Radiopharma, Germany), ITLC-SG paper (Agilent Technologies, California), ITLC chambers, Automated Gamma Counter (Wizard 2470Perkin Elmer, UK).

Preparation of DTPA kit

The vials and glassware were heated using the oven with a temperature set to 250ºC for 3 hours for depyrogenation. Heat-labile apparatuses such as rubber stoppers were steam sterilized using autoclave temperature set to 121ºC for 30 min. Water for injection (WFI) was bubbled with nitrogen gas to be used throughout the procedure. 15.0 mg of Stannous Chloride powder was dissolved in the mixture of 100 µL 10M HCl followed by an additional 5 mL of water for injection. Then, the solution was shaken vigorously by using the vortex. 515.0 mg DTPA was dissolved in 2 mL 2M NaOH. A volume of 2 mL WFI was added to the reaction mixture. OFN gas was channelled into the reaction mixture to ensure complete removal of oxygen from the reaction mixture before adding 2.55 mL stannous chloride solution from the stock by using a micropipette. A volume of 1 M HCl was gradually added into the reaction mixture until the pH of 3.9 - 4.1 was obtained. Using a 25 mL volumetric flask, water for injection was added into the reaction mixture until it reached the calibration mark. The reaction mixture was then being filtered out into a vial by using a 0.22 µm filter. The sealed final volume of the formulation was taken to the cleanroom for dispensing by using the aseptic technique. The filtered reaction mixture was aliquoted into 25 separate sterile evacuated vials (SEV) containing 1 mL for each vial. The vials were freeze-dried into the freeze dryer using the following condition as in Table 2-1

Table 2-1. Freeze drying condition

Freeze temperature	Eutectic temperature	Dried temperature	Time
-30ºC	-1ºC	24ºC	24-48 h

Certificate of Analysis (CoA) of DTPA kit

The working condition was monitored by an appropriate sampling of the working area. Two types of media were exposed to the open air during the process near the working station. A fluid thioglycollate medium was used mainly to culture if there was the presence of anaerobic bacteria. Another type of media that was used during the process was the Soya-bean Casein digest medium. This medium is suitable for culture if there was the presence of fungi and aerobic bacteria. After the procedure was completed, the media and DTPA kit was sent to the Quality Control Unit of Malaysian Nuclear Agency for the sterility, bacterial endotoxin test. Another quality control test to acquire the certificate of analysis of DTPA kit was the appearance of the solution, pH, radiochemical purity (RCP) and stannous content. The method that was used to analyze the stannous content was by polarography method.

Radiolabelling of ^{99m}Tc-DTPA

^{99m}Tc solution was obtained from ^{99}Mo/^{99m}Tc Generator (France) for radiolabelling of DTPA kit. A pure ^{99m}Tc solution was used to label the DTPA kit. The DTPA kit was removed from the refrigerator and thawed to room temperature. The radiolabelling process was done by adding 1 mL of pure ^{99m}Tc into the vial containing the DTPA kit. Next, the mixture was vortexed for 30 seconds and incubated at room temperature for 15 minutes.

Instant Thin layer Chromatography (ITLC)

Next, saline and acetone were prepared in separate ITLC chambers. Saline and acetone were the mobile phases, while silica gel on the ITLC strips was stationary. The impurities that can be determined from the saline solvent system are reduced hydrolyzed ^{99m}Tc while free ^{99m}Tc-pertechnetate can be determined from the acetone solvent system. 3 µL of the radiolabelled ^{99m}Tc-DTPA complex was spotted on the origin. The strip was placed gently in the chamber, and the solvent was allowed to develop the strips. When the solvent reached the solvent front, the strip was removed from the chamber and let to dry in the oven. The strip was then measured its percentage of impurities corresponding to its area under the curve of the dedicated peak over the totalarea under the curve in one strip by using ® AR-2000 Radio-TLC Imaging Scanner (Eckert & Ziegler Radiopharma, Germany).

Finally, the percentage of the RCP of the radiolabelled complex was determined by using Equation 2-1

Equation 2-1. Calculation of the RCP of ^{99m}Tc-DTPA

$$\%\ ^{99m}\text{Tc-DTPA} = 100\% - \text{reduced hydrolyzed } ^{99m}\text{Tc } (\%) - \text{free } ^{99m}\text{Tc-pertechnetate } (\%)$$

In vitro stability study of ^{99m}Tc-DTPA

The stability of ^{99m}Tc-DTPA was studied in vitro by incubating the complex at room temperature alone and by mixing with 100 µL of 5% Human Serum Albumin (HSA) in the complex. The complexation yield was measured using the ITLC method at different time points.

^{99m}Tc-DTPA imaging

the Sprague-Dawley rat was anaesthetized by using isoflurane. The rat was then injected with ^{99m}Tc-DTPA and quarantined in the metabolic cage. When the time point was reached, the rat was scanned by using a gamma camerato see the distribution of the product in the rat at six different time points within the range of 5 minutes up to 24 hours. The image was compared

with the negative control,which is free ^{99m}Tc.

In vivo Biodistribution studies

About 1-1.5 mCi radioactivity in a 200 µL sample was injected into the tail vein of rats, and the rats were then quarantined. When the time point was reached, the rat was anaesthetized using isoflurane and being euthanized by using cardiac puncture and cervical dislocation technique. The organs of the rats were collected in the test tube. The test tubes were being weighed and counted the radioactivity of each organ by using a gamma counter. The result was then compared to the control, which is free ^{99m}Tc intravenously injected into the Sprague-Dawley rats. The percentage values of the injected dose per gram organ (%ID/gm ± SD) in a population of 3 rats for each time point were then calculated using the formula in Equation 2-2

These studies have been further approved by CUCMS Animal Care and Use Committee (CACUC), and animal care and procedures followed are in accordance with the guidelines and license issued by the university (Registration numbers: CACUC/1/2020/2).

$$\%\ \text{ID/ gram organ or tissue} = \frac{\%\ \text{Radioactivity of tissue or fluid}}{\text{Weight of tissue/fluid}}$$

Equation 2-2. %ID/gram organ or tissue

RESULTS AND DISCUSSION

DTPA kit analysis

The importance of DTPA kit analysis is to acquire the Certificate of Analysis (CoA) and ensure the safety of the DTPA kit to be injected into the patient's body. The tests included in the study are the appearance of the DTPA kit solution, pH, radiochemical purity (RCP), stannous content, sterility, and bacterial endotoxin test. The specification and the result of the analysis were shown in Table 3-1

Table 3-1. Analysis of DTPA kit

	TEST	SPECIFICATION	RESULTS
1.	Appearance of solution	Clear and colourless	Clear and colourless
2.	pH	5.0 – 7.5	6.5
3.	Radiochemical Purity(RCP)	> 95 %	97.53
4.	Stannous content ($SnCl_2.2H_2O$), (mg)	> 50 % of $SnCl_2.2H_2O$ content (0.3 mg) as claimin the formulation	0.1854
5.	Sterility	Sterile	Sterile
6.	Endotoxin (EU/ml)	<17.5 EU/ml	PASS

Based on Table 3-1, the appearance of the solution was clear and colourless. This indicates the solution of the DTPA kit after radiolabelled with the ^{99m}Tc was free from debris or suspended particles that are insoluble in the solution. Injecting radiopharmaceutical that contains suspended particles is harmful to the patient as it may produce medical complications such as blockage of small blood vessels, leading to ischemia [6]. The optimum pH of 6.5 for radiopharmaceutical is important because the unsuitable pH value of the radiopharmaceutical solution would affect the stability of the radiolabelled complex [3]. High RCP of the radiolabelled complex indicates that most isotopes are attached to the ligand and are not free or attached to another chemical entity, which may affect the biodistribution of the complex in the body. Low RCP could interfere with diagnostic scanning by obscuring the region of interest and impeding the scan interpretation [7]. The presence of stannous ion in the kit is crucial to reducing the oxidation number of ^{99m}Tc from +7 state to a lower number and enabling it to be attached effectively to the DTPA. Stannous content might lose from the final preparation because stannous ions are highly susceptible to oxidation when exposed to the atmosphere during the procedure or dissolved oxygen in the solution [8]. In the freeze-drying procedure, the vacuum in the vials was preserved during stoppering step to prolong the shelf-life of the kit up to one year. The DTPA kit in this study was passing the sterility, and apyrogenicity tests show it is safe to be administered to the patient.

Instant Thin Layer Chromatography (ITLC)

Table 3-2. ITLC result in the acetone solvent system

	Rf	Percentage (%)
^{99m}Tc-DTPA + reduced hydrolyzed ^{99m}Tc	0.2	99.200 ± 0.021%
Free ^{99m}Tc-pertechnetate	1.0	0.800 ± 0.021%

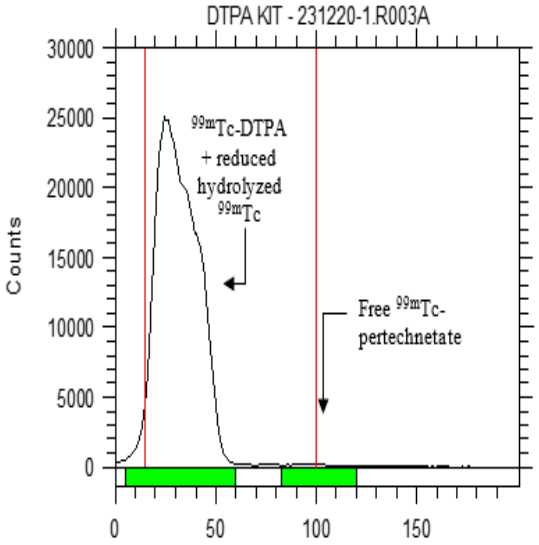

Figure 3-1. Separation graph of 99mTc-DTPA in acetone solvent system

Table 3-2 and Figure 3-1 show the result of the ITLC of ^{99m}Tc-DTPA in the acetone solvent system. The Rf value of the ^{99m}Tc-DTPA complex was 0.2 and not being separated from the reduced hydrolyzed ^{99m}Tc. However, the complex was separated from free ^{99m}Tc-pertechnetate, which has an Rf value of 1.0. Figure 3-1 shows the complex and reduced hydrolyzed ^{99m}Tc share the same peak near the origin with a largearea under the curve, corresponding to 99.200 ± 0.021 %. Towards the solvent front of the ITLC strip, the peak of free ^{99m}Tc-pertechnetate has a very small area under the curve corresponding to 0.800 ± 0.021%. This is because free ^{99m}Tc-pertechnetate is a small ion that migrates quickly with the solvent and separates easily with the larger complexes. Apart from that, free ^{99m}Tc-pertechnetate exists in the solvent system as an anion molecule. therefore, due to its solubility of anion in the polar solvent such as normal saline and acetone, free ^{99m}Tc-pertechnetate can easily migrate with the solvent to the solvent front [9].

Table 3-3. ITLC result in saline solvent system

	Rf	**Percentage (%)**
Reduced hydrolyzed ^{99m}Tc	0.2	0.540 ± 0.113%
^{99m}Tc-DTPA + free ^{99m}Tc-pertechnetate	1.0	99.460 ± 0.113%

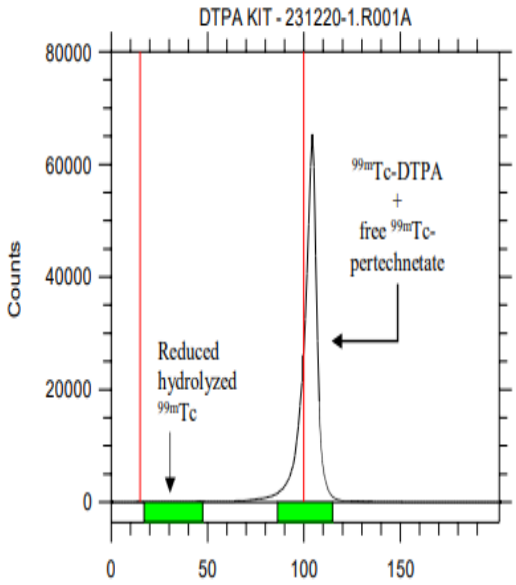

Figure 3-2. Separation graph of 99mTc-DTPA in saline solvent system

Table 3-3 and Figure 3-2 show the ITLC of ^{99m}Tc-DTPA in the saline solvent system. Reduced hydrolyzed ^{99m}Tc, which has an Rf value of 0.2, was separated from ^{99m}TcDTPA complex. However, free ^{99m}Tc-pertechnetate was not separated from the complex, which has an Rf value of 1.0. Figure 3-2 shows the peak of the reduced hydrolyzed ^{99m}Tc near the origin has a tiny area under the curve corresponding to 0.540 ± 0.113%. The peak of the ^{99m}Tc-DTPA and the free ^{99m}Tc-pertechnetate was shifted to the right, which indicates it near the solvent front of the ITLC strip has a large area under the curve corresponding to 99.460 ± 0.113%. due to the chemical properties of ^{99m}Tc-DTPA, which is very soluble in the polar solvent (normal saline), it can join free ^{99m}Tc-pertechnetate migrate to the solvent front, which gives their Rf value of 1.0 while reduced hydrolyzed ^{99m}Tc remains at the origin spot which gives its Rf value of 0.2. the Rf value of these compounds depends on the dielectric constant or the polarity of the mobile phase [9]. Based on the ITLC result of the ^{99m}Tc-DTPA in both solvent systems, the RCP percentage of the radiolabelled complex was calculated as in Equation 3-1

$$\text{RCP of } ^{99m}\text{Tc-DTPA} = 100\% - (0.800 \pm 0.021\%) - (0.540 \pm 0.113\%)$$
$$= 98.600 \pm 0.092\%$$

Equation 3-1. The RCP of 99mTc-DTPA

In vitro stability study of ^{99m}Tc-DTPA

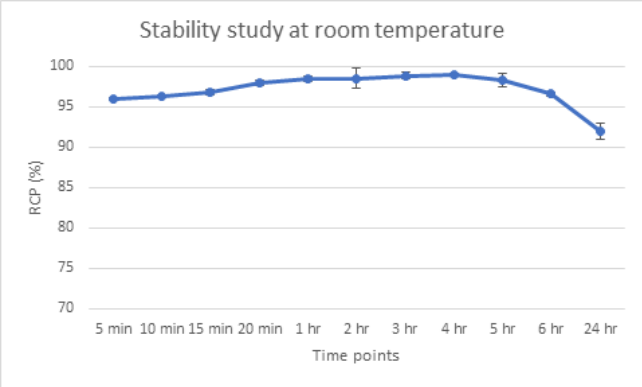

Figure 3-3. Stability study of 99mTc-DTPA at room temperature

The acceptable RCP value of the ^{99m}Tc-DTPA as stated by IAEA is above 95%. Based on Figure 3-3, at 5 minutes, the complex achieved an acceptable RCP value of 95.960 ± 0.191%, and the percentage of RCP was increasing gradually until it reached peakRCP at 4 hours with 98.950 ± 0.134%. The complex was stable for up to 6 hours with 96.570 ± 0.283% of RCP. However, at 24 hours, the RCP of ^{99m}Tc-DTPA indicated as91.950 ± 1.018%.

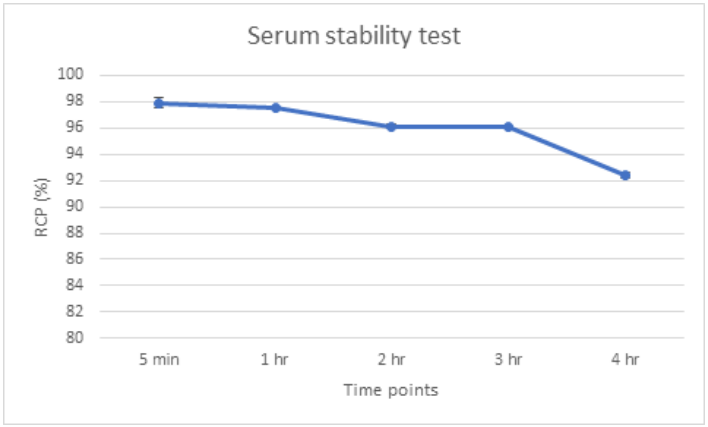

Figure 3-4. 99mTc-DTPA serum stability study

Based on Figure 3-4 at 5 minutes, the complex achieved an acceptable RCP value of 97.920 ± 0.396%. The RCP of the complex was stable for up to 3 hours with 96.070 ± 0.035% before falling below 95% at 4 hours with RCP of 92.460 ± 0.226%.

The stability of the complex in the HSA is expected to be lower than when incubated alone at room temperature because all radiopharmaceuticals are susceptible to some degree of weak affinity to some component of the blood such as serum albumin and α-1 acid glycoprotein. The most common interaction occurs between the radiopharmaceutical and the blood component, which is hydrogen-bonding interaction that causes the degradation of the complex and reduces its stability [10], [11]. The stability of the complex in the final preparation and human serum proved to be enough to allow for any medical procedure. These results also will enable the radiolabelling of DTPA kit with ^{99m}Tc early in the morning and can be used up until afternoon (6 hours).

^{99m}Tc-DTPA imaging

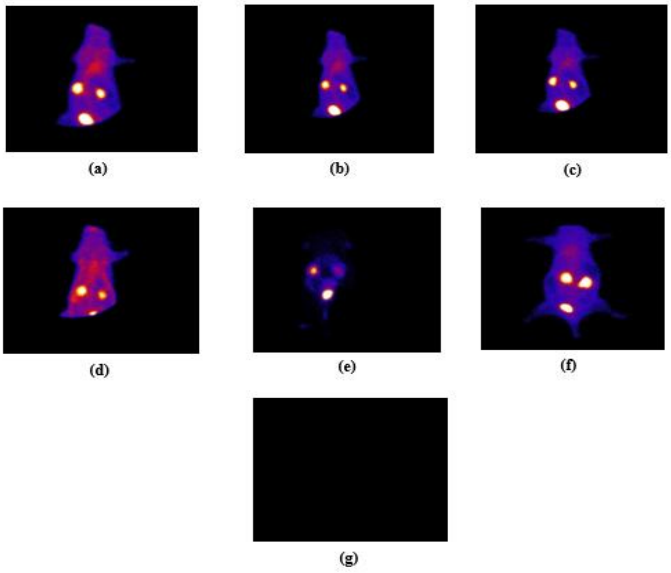

Figure 3-5. Distribution of 99mTc-DTPA in the Sprague Dawley rat (a) Distribution at 5 minutes (b) Distribution at 5 minutes (c) Distribution at 30 minutes (d) Distribution at 1 hour (e) Distribution at 2 hours (f) Distribution at 4 hours (g) Distribution at 24 hours

The hot areas shown by the fluorescent areas are the rat's organ that has the accumulation of the radionuclide. Figure 3-5 shows a distribution of the ^{99m}Tc-DTPA in both left and right kidneys and the rat's bladder. At 5, 15, 30 minutes and 1 hour, the accumulation of the ^{99m}Tc-DTPA was high in the kidney, but there was slight distribution at other parts of the body, especially at the heart area. At 2 and 4 hours, the accumulation of ^{99m}Tc-DTPA is the highest at the kidneys. The distinct image of the kidneys was obtained with minimal distribution at other parts of the body apart from the bladder. At 24 hours, the hot area was not visible at any part of the body showing that the ^{99m}Tc-DTPA was completely cleared from the body. Based on the literature, 96% of injected doses would be cleared from the body after 24 hours of injection [12]. In the clinical setting, the patient is scanned under a gamma camera after being injected with ^{99m}Tc-DTPA (15 millicurie (mCi) for adults and 0.1-0.2 mCi/kg for children) to evaluate renal blood flow through serial dynamic imaging [13]. Based on the Clinical Guideline for the measurement of glomerular filtration rate (GFR) using plasma sampling, The GFR of the patient can be determined by blood sampling as early as 2 hours after the injection depending on the Body Surface Area (BSA) of the patient [14]. In this study, the most apparent uptake of the complex in the kidneys was seen at 2- and 4-hours post-injection with minimal distribution to another organ. This result enables the actual patient to be tested for GFR 2 or 4 hours after being injected with ^{99m}Tc- DTPA to get a more accurate result.

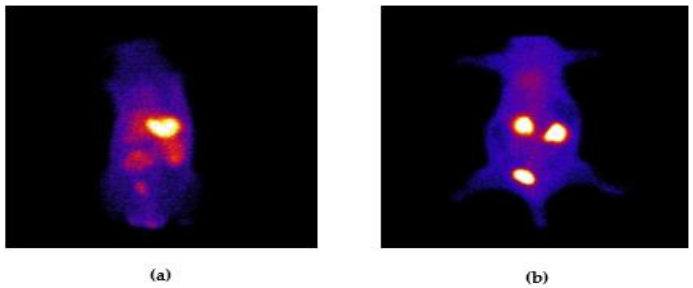

Figure 3-6. Distribution of the sample at 4 hours (a) Distribution of the free 99mTc (b) Distribution of the ^{99m}Tc-DTPA

Figure 3-6 shows the comparison of the distribution between free ^{99m}Tc and ^{99m}Tc- DTPA in the rats at 4 hours post-injection. The hot area of the rat that was injected with free ^{99m}Tc is randomly scattered in the body. It was highly accumulated in the stomach, which has the brightest hot area and slight accumulation of the sample at the kidneys and the bladder. However, in the rat injected with ^{99m}Tc-DTPA, the distribution of the complex is more specific and highly accumulated in the kidney and the bladder of the rats. Based on the literature, injecting free ^{99m}Tc alone into the rat shows high uptake in the stomach and other parts of the body such as the thyroid and salivary glands [15].

In vivo Biodistribution studies

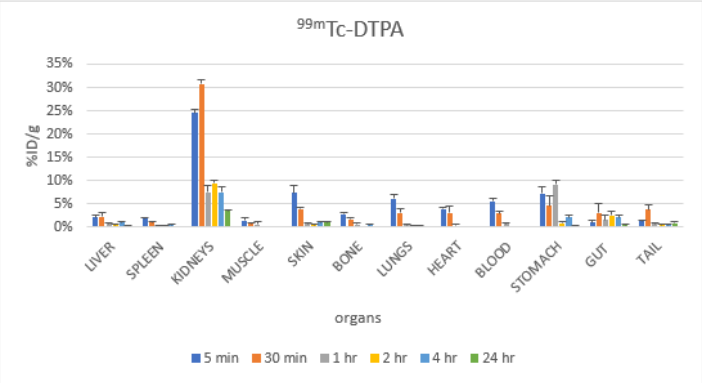

Figure 3-7. Percentage of injected dose per gram organ (%ID/g) of 99mTc-DTPA at various time interval

Based on Figure 3-7, the biodistribution study in the Sprague Dawley rat indicated that 24.489 ± 0.008% of the injected dose (%ID/g) accumulated in kidneys at 5-minutes post-injection. The uptake of the radioactive in the kidneys was slightly increased to 30.766 ± 0.010% at 30 minutes before drastically decrease to 7.545 ± 0.013% at 1 hour. This is because more than 90% of the injected activity of ^{99m}Tc-DTPA was washed out via kidneys through glomerular excretion within 2 hours, and only 10% of it bound to the plasma protein [8]. The kidneys' uptake was gradually decreased over the time at 2 hours until 24 hours post-injection. There was some distribution in the skin, lungs, heart, blood, and stomach at 5 minutes and 30 minutes but remain lower than the percentage of injected dose per gram organ (%ID/g) shown by the kidney. The slight uptake of ^{99m}Tc-DTPA in the blood at 5 min (5.588 ± 0.007%ID/g) and 30 min (3.030 ± 0.004%ID/g) was due to the weak affinity of the complex towards the blood components, which affect the radiopharmaceutical distribution to the blood. ^{99m}Tc-DTPA is a low protein-bound radiopharmaceutical. Therefore, it is likely to cross the capillary membrane into the tissue during the short residence time of blood in capillaries [10].

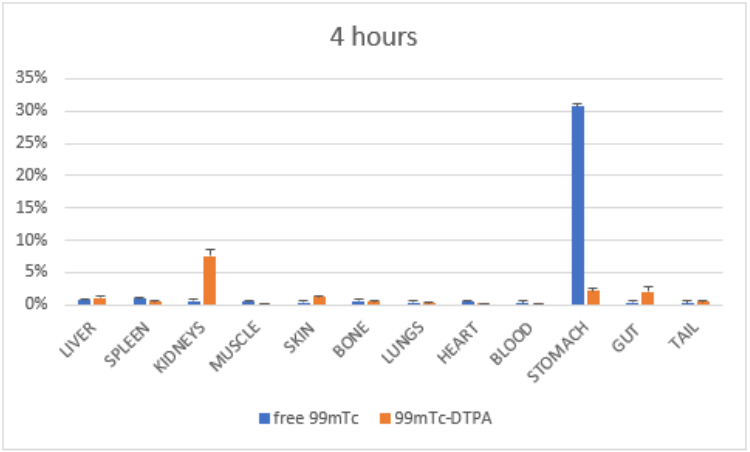

Figure 3-8. Percentage of injected dose per gram organ (%ID/g) of the samples at 4 hours

Figure 3-8 summarised the percentage of injected dose per gram organs (%ID/g) for biodistribution studies of all samples at 4 hours. The rats injected with free ^{99m}Tc have the highest accumulation in the stomach with 30.755 ± 0.003%, while the rats that injected with ^{99m}Tc-DTPA has the highest accumulation in the kidneys with 7.596 ± 0.010%. However, there was slight uptake in the stomach and gut for the ^{99m}Tc-DTPA, and the uptake of the free ^{99m}Tc in the kidney was almost negligible. Statistical analysis using independent t-test shows that there was a significant difference between the percentage of injected dose per gram organ (%ID/g) in the kidney between free ^{99m}Tc (0.625 ± 0.444%ID/g) and ^{99m}Tc-DTPA (7.596 ± 1.430%ID/g) with $p < 0.05$.

CONCLUSION

The ^{99m}Tc-DTPA prepared in this study is a safe and effective for the diagnostic and imaging of renal. The certificate of analysis acquired in this study proves the DTPA kit are safe to be used in the patients of kidney disease in which the imaging is necessary. The use of ^{99m}Tc as a radiotracer is appropriate and harmless to the patients. The final preparation demonstrates that it can be radiolabelled with ^{99m}Tc with RCP up to 98.660 ± 0.060%. The preparation of ^{99m}Tc-DTPA was stable at room temperature up to 6 hours and in HSA up to 3 hours. The distribution of ^{99m}Tc-DTPA in the body, as shown in the ^{99m}Tc-DTPA imaging study and biodistribution study, is

localised in the kidney suitable with its indication for renal scintigraphy. The biodistribution study also showed that the clearance of ^{99m}Tc-DTPA has rapid excretion through kidneys, and almost complete dose is cleared from the body by 24 hours. The usefulness of this ^{99m}Tc-DTPA in the clinical setting can be seen because it can be radiolabelled on-site since it can be obtained through ^{99}Mo/^{99m}Tc generator. The stability of the complex at room temperature for up to 6 hours makes it easier for the radiopharmacist to prepare it in the morning and use it until the afternoon. This method also is the most accurate method to determine the GFR of the specific patient compared to other methods.

Funding: This research was funded by University of Cyberjaya and Yayasan Peneraju Pendidikan Bumiputra

Conflicts of Interest: The authors declare no conflict of interest and the funder had no role in the design of the study; in the collection, analyses, or interpretation of data; and in the writing of the manuscript

REFERENCES

i. Erwin, W. D. (2013). Physics in Nuclear Medicine. In *Journal of Nuclear Medicine* (Vol. 54, Issue 7). https://doi.org/10.2967/jnumed.113.123125

ii. Mititelu, R., & Bratu, O. (2017). Radionuclide Imaging. An Update on the Use of Dynamic Renal Scintigraphy. Medicina Moderna - Modern Medicine, 24(4), 199– 203. https://doi.org/10.31689/rmm.2017.24.4.199

iii. Saha G.B., (2018). Fundamentals of Nuclear Pharmacy (7th ed.). New York, United State, America: Springer.

iv. Rathmann S.M., Ahmad Z., Slikboer S., Bilton H.A., Snider D.P., Valliant J.F. (2019) The Radiopharmaceutical Chemistry of Technetium-99m. In: Lewis J., Windhorst A., Zeglis B. (eds) Radiopharmaceutical Chemistry. Springer, Cham. https://doi.org/10.1007/978-3-319-98947-1_18

v. El-gizawy, M. A. E. (2013). Comparative Studies on the Radiolabeling and Chromatographic Purification of Some Medically Important Compounds.

vi. McLean, S., Patel, R., & Bruno, R. (2017). Injection of Pharmaceuticals Designed for Oral Use: Harms Experienced and Effective Harm Reduction Through Filtration. *Current topics in behavioral neurosciences*, *34*, 77–98. https://doi.org/10.1007/7854_2016_470

vii. Maltby, P. (2009). Radiopharmacy quality control. *European Association of Nuclear Medicine*, *CTE*.

viii. Amin, M. R., Haque, A., Biswas, A., & Mozumder, T. H. (2014). Preparation and labeling of technitium-99m kit In pharmaceutical grade clean room. *Journal of Chemical Engineering*, *27*(2), 31–35. https://doi.org/10.3329/jce.v27i2.17798

ix. Putra, A. R., Lestary, E., Maskur, M., & Tahyan, Y. (2020). Validation of [99m Tc]Tc-DTPA radiochemical testing method using one-system paper chromatography . *Journal of Physics: Conference Series*, *1436*, 012001. https://doi.org/10.1088/1742-6596/1436/1/012001

x. Berridge, M. S. (2009). The importance of kinetic enhancement. *Journal of Nuclear Medicine*, *50*(8), 1203–1204. https://doi.org/10.2967/jnumed.108.060905

xi. Ahmed, H., Zimmer, M., Cutrera, P., McKay, B., Riehle, L., & Spies, S. (2015). Radichemical Purity and Stability of Generic Tc-99m DTPA. *Journal of Nuclear Medicine*, *56*(supplement 3), 2740-2740.

xii. Alsabea, H. (2017). Type of Renal Scintigraphy. *Journal of Nuclear Medicine & Radiation Therapy*, *08*(06), 2–9. https://doi.org/10.4172/2155-9619.1000347

xiii. Banker H, Sheffield EG, Cohen HL. Nuclear Renal Scan. [Updated 2020 Sep 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available

xiv. Burniston, M. (2018). *Clinical Guidelines for the measurement of glomerular filtration rate (GFR) using plasma sampling*. 23.

xv. https://www.bnms.org.uk/images/BNMS_GFR_guidelines_in_BNMS_template_format_FINAL.pdf%0Ahttps://cdn.ymaws.com/www.bnms.org.uk/resource/resmgr/guidelines/bnms_gfr_guidelines_in_bnms_.pdf

xvi. Franken, P. R., Guglielmi, J., Vanhove, C., Koulibaly, M., Defrise, M., Darcourt, J., & Pourcher, T. (2010). Distribution and dynamics of ^{99m}Tc-pertechnetate uptake in the thyroid and other organs assessed by single-photon emission computed tomography in living mice. *Thyroid*, *20*(5), 519–526. https://doi.org/10.1089/thy.2009.0213