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PHYSICOCHEMICAL CHARACTERIZATION OF ^{99m}TECHNITIUM - LUTEOLIN AS RADIOPHARMACEUTICAL PREPARATION FOR ANTIOXIDANT COMPOUND

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ABSTRACT: The objective of this study was to formulate luteolin with radioisotope Technetium-99m (^{99m}Tc) to be ^{99m}Tc-Luteolin as an antioxidant compound. After optimization, this radiopharmaceutical preparation should be examined for its physicochemical characteristics, such as radiochemical purity, stability, lipophilicity, protein plasma binding, electronic charge, and stability test. The formulation was done by mixing the luteolin compound with radioisotope ^{99m}Tc by using $SnCl_2$ as a reducing agent. Radiopharmaceutical compound ^{99m}Tc-Luteolin was formed, then tested for physicochemical characteristics. The result of radiochemical purity was 98.19% ± 1.30%, Log P 0.95 ± 0.07, protein plasma binding 38.01% ± 4.28%, and had neutral charges, test stability at room temperature showed that the radiopharmaceutical purity value was better than in plasma. The results obtained in this research work indicated ^{99m}Tc-Luteolin radiopharmaceutical compounds had good physico-chemical properties and were relatively stable in room temperature than in blood plasma. This optimization condition has the potential to be developed into a radiopharmaceutical kit.

INTRODUCTION: Free radical is an unstable and reactive compound which has one or more free electron. This compound can cause harm to the body, called oxidative stress if presented too much and accumulated. Oxidative stress occurs when there's an imbalance between free radical productions and antioxidant amount. This condition will cause cell damage which leads to more disease occurrence [1].

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Antioxidant, meanwhile, is a stabilizing agent for free radicals so that those won't attack or damage body cells. The antioxidant can be produced within the body or called as an endogenous antioxidant, or comes from outside the body, called exogenous antioxidant. This exogenous antioxidant may come from foods such as vegetables and fruits, or other dietary supplements [2].

Luteolin is an exogenous antioxidant used in this research. Luteolin is a flavonoid contained in vegetables, fruits, or herbal plants that acts as antioxidant and cancer preventive agent. Luteolin is much contained in celery, parsley, broccoli, leek, carrot, pepper, cabbage, apple skin, and chrysanthemum. Luteolin has been reported to have an anti-cancer characteristics [3].

^{99m}Technetium labeled radiopharmaceutical is a preparation used to diagnose or treat certain diseases. This radionuclide was selected because of its short half-life, which is 6.01 h, has relatively lower energy emission (141 KeV), and radiates gamma rays which helps diagnose disease [4]. ^{99m}Tc-Luteolin radiopharmaceutical is expected as diagnosing and therapeutic agent for oxidative stress. Luteolin is not known exactly to what part of the body; this compound collects it. Characterization and quality control of radiopharmaceutical have to be done to know its physicochemical properties. Later, from those properties, the pharmacokinetic properties such as organ distribution, can be predicted.

This research aims at knowing the physicochemical properties of radiopharmaceutical such as radiochemical purity, stability, lipophilicity, and protein plasma bonding [5]. By knowing its physicochemical properties and other technical data, it can be useful in ^{99m}Tc-Luteolin radiopharmaceutical use.

MATERIALS AND METHODS:

Materials: Equipment used in this research were electrophoresis instrument, dose calibrator (Victoreen®), micropipette (5 µl, 10-100 µl, 20 - 200 µl, 200-1000 µl, 1000 µl) (Eppendorf®), analytic scale (Mettler Toledo® Type AL 204), oven (Memmert®), centrifugation (Fisher®), Single Channel Analyzer (SCA) (ORTEC®), syringe 1 ml dan 6 ml (Terumo®), centrifugation tube, vial 10 ml, Thin Layer Chromatography (TLC) scanner BIOSCAN AR2000, and vortex (Victoreen®).

Materials used in this research were Luteolin (Solistree®), ammonium hydroxide (Merck®), aquadest, aquabidest (IKA Pharma®), dimethyl sulfoxide (DMSO) (Merck®), ethanol absolute (Merck®), HCl 0.1 N (Merck®), human blood plasma, Instant Thin Layer Chromatography-Silica Gel (ITLC-SG) plate (Agilent Technologies®), Thin Layer Chromatography, Na ^{99m}TcO4 (Ansto), NaCl saline solution (IKA Pharma®), NaOH 0.1 N (Merck®), n-octanol (Merck®), phosphate buffer solution 0.02 N pH 7.5; $SnCl_2.2H_2O$ (Sigma Aldrich®), universal pH indicator (Merck®), and Whatman 1 paper. All other ingredients used were of analytical grade.

Methods:

Radiochemical Purity Test: Purity test was done by paper chromatography method. Papers used for this test were KLT-SG_{F254} and ITLC-SG. Meanwhile, NaCl saline solution and C1 – the mixture of ethanol: water: ammonia (2:5:1) were used as the eluents for both paper respectively. The sample was tipped in the paper plate by micropipette then eluted. The plate then dried in the 80 °C oven for 10-15 min. Wrap the plate with tape so that it won't scattered [7]. The papers were cut in every 1 cm length, then analyzed in single channel analyzer. The result counted with the equation below [7].

$$\%\ ^{99m}\text{Tc-luteolin} = 100\% - (\%\ ^{99m}TcO_2 + \%\ ^{99m}TcO_4^-)$$

Stability Test:

Stability Test in Room Temperature: This test was done by observing physical changes on every hour for 5 h. The radiochemical purity was counted to observe the chemical changes in the preparation on the same interval as physical observation, by the same method as radiochemical purity test [6].

Stability Test in Blood Plasma: *In-vitro* stability test was done in human blood plasma. After radiochemical purity was achieved, 250 µl sample added to 500 µl blood plasma. Then vortexed for 1 min to homogenize them. The mixture then incubated in 37 ºC. The radiochemical purity of sample in the blood plasma was counted after 15, 30, 45 min, and 1, 2, 3, 4, 5 h incubation [6].

Lipophilicity Test: This test followed the partitioning principle of the sample in two different polarity solvent – n-octanol and water (NaCl saline solution was used). Each 1 ml solvent added to the centrifugation tube then added by 10-50 µl sample. Then vortexed for 1 min. After that, the mixture was centrifuged in 3000 rpm for 10 minutes. After both phases separated, take 50-100 µl of each phase to be counted in single channel analyzer. The obtained counts then counted by the equation below.

Partition coefficient = lipophilicity (P) = octanol phase counts / NaCl saline phase counts

That log P value defined the lipophilicity of ^{99m}Tc-Luteolin radiopharmaceutical. The remaining octanol phase then moved into a new tube then added again by NaCl saline solution of the same

volume. Repeat the mixing and separating steps until a relatively constant value was reached.

Protein Plasma Binding Test: 500 µl blood plasma added to centrifugation tube. Then 50 µl sample added. Then vortexed for 1 min. The mixture then incubated at 37 °C for 10 min. After that, 1 ml of NaCl saline solution and 1 ml of TCA 5% added to the mixture. The mixture then vortexed once again then centrifuged with 300 rpm for 15 min. After that, the supernatant and precipitate were separated.

In the supernatant, 1 ml of TCA 5% was added. Then the precipitating and separating process was repeated. The same repetition also applied to precipitation phase. 1 ml of saline solution added to that as a washing. The supernatant and precipitation of each tube then separated again and each counted in single channel analyzer. The protein plasma binding value was obtained from the equation below

Protein plasma binding (%) = Precipitation counts × 100% / Total counts (Precipitation + Supernatant)

Electric Charge Test: The electric charges determination was carried out by paper electrophoresis. Whatman 1 paper, as stationary phase was cut into 37 cm × 1 cm size. Then it was numbered at every 1 cm length, from -18 to +18. Then the paper was placed in the electrophoresis chamber with the number 0 in the middle, the negative number on the cathode and the positive number on the anode. Prior to running, the paper wetted with phosphate buffer solution 0.2 N pH 7.5. Then the sample tipped to the "0" area.

Electrophoresis chamber then closed and 350-volt voltage and 11-16 mA electricity given to run the process. The running process took 45 min then after that, the paper dried in the oven. Every 1 cm paper counted in single channel analyzer and migration curve is composed based on the obtained counts [5].

RESULTS AND DISCUSSION: Radio-pharmaceutical has strict quality control, also designated additional tests besides the common ones, which were radiochemical purity test, labeling effectiveness, and biological test [8]. In this research, the biological test was excluded.

Luteolin was a new compound isolated from the daily consumed fruits and vegetables [3]. It was selected as the active component for this radiopharmaceutical for its antioxidant activity. From its structural point, Luteolin had hydrophilic functional groups [9] that could donate a proton to neutralize the threatening free radicals [10]. On the other hand, Technetium-99m was used as a radioisotope for its gamma ray, lower free energy, and the ability to pass through tissues. Other than that, Tc^{99m} has a short half-life [4] which was relatively safer than other radioisotopes. Luteolin, as anti-free radical, was capable of pulling free radical into its structure. According to Oganesyan and Tvorovskii [10], flavon derivates were capable of binding –OH free radicals to its conjugated group C=C, $C^2=C^3$ **Fig. 1**. These groups were more reactive to free radicals because it required lower activation energy and might have addition reaction. Moreover, the higher electron density in these groups made the free radical which was electrophile, attracted (see **Fig. 1**).

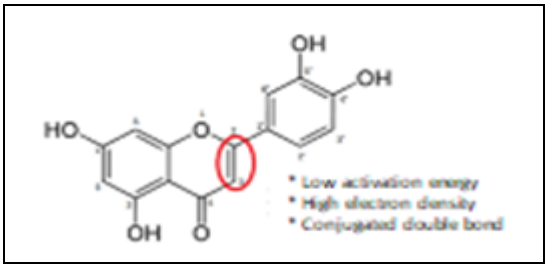

FIG. 1: BINDING PREDICTION OF FREE RADICALS IN LUTEOLIN

Radiochemical Purity Test Result: Radio-chemical purity was tested to know whether labeling process was successful or not. The high purity percentage defines fewer contaminant. A contaminant will present if the redox reaction on technetium did not go properly. Technetium-^{99m} usually presents in 7^+ valence. This number couldn't bind and complex with another compound, including luteolin, properly. Because of that reason, technetium had to be reduced from 7^+ to 4^+ before reacted. The help from a reducing agent was needed. The reducing agent used in this research was $SnCl_2$[7]. Among reducing agent, $SnCl_2$ was selected because of its nontoxic and moderate reducing activity. The following is the reaction between $SnCl_2$ and TcO_4.

$$2Sn^{2+} \leftrightarrows 3Sn^{4+} + 6e^-$$

$$2^{99m}TcO_4^- + 16\ H^+ + 6e^- \leftrightarrows 2^{99m}Tc^{4+} + 8H_2O$$

$$2^{99m}TcO_4^- + 16\ H^+ + 3Sn^{2+} \leftrightarrows 2^{99m}Tc^{4+} + 8H_2O$$

The contaminants of this reaction, TcO_2 and TcO_4 were counted to quantify the radiochemical purity of the preparation. Those two could be detected by chromatography method. There were two plates used for the test, KLT SG_{F254} and ITLC-SG with NaCl saline solution and C1 (ethanol: water: ammonia = 2:5:1) as eluents. NaCl saline solution was able to separate TcO_4 with the desired complex (^{99m}Tc-Luteolin) with R_f value 0 [4], meanwhile, C1 could separate ^{99m}Tc-Luteolin from TcO_2 with R_f value 1 [8, 9, 10]. These contaminants could be analyzed by single channel analyzer or TLC scanner. The result of the purity test was 98.19% ± 1.62%. According to FDA and USP, this value could be accepted. The purity of radiochemical should have 90% or more in value.

Stability Test Result:

Stability Test in Room Temperature Result: This test is important to do because stability can affect the effects of the radiopharmaceutical in the body. Stability depends on the radiochemical purity value in few hours after incubation period ended [11, 12].

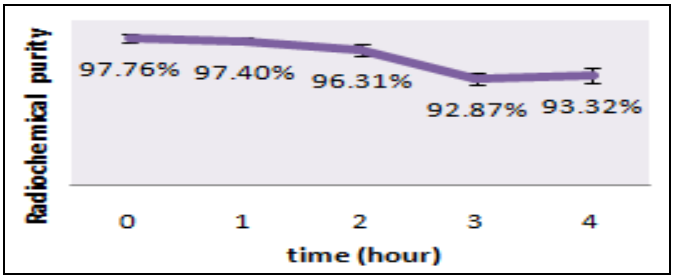

FIG. 2: GRAPH RESULT OF STABILITY IN ROOM TEMPERATURE

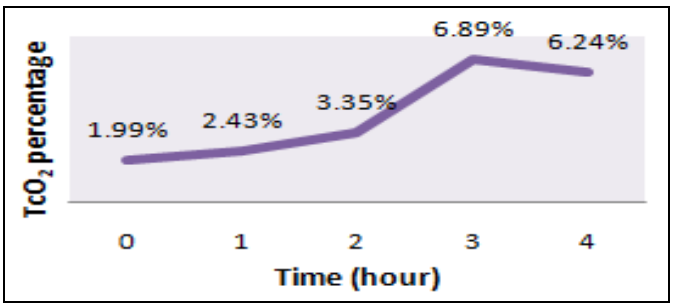

FIG. 3: ELEVATION OF TcO_2

In-room temperature stability, the result is shown in **Fig. 2** indicated that the tested radiopharmaceutical lasted up to 2 h after incubation because the purity dropped below 95% and physically changing. The unclear solution might be happened because of TcO_2 elevation which was a colloid and couldn't be dissolved in water. Graph of TcO_2 elevation shown in **Fig. 3**. This test showed that the storage time of the formulated preparation was 2 h after incubation.

Stability Test in Blood Plasma Result: This test was especially done for radiopharmaceutical formulated as intravenous injection preparation. Drugs which were administered in blood will interact with blood components, especially blood plasma component [5]. The interaction between blood plasma components and the tested radio-pharmaceutical should be studied to know the stability and pharmacokinetic profile. Blood plasma consists of many components such as protein, enzyme, water, and salts [13]. Things that enter the body, including drugs, have to reach the desired target and for that, blood plasma should transport them. This was the reason why blood plasma stability should be assured. If drugs were unstable and experience some changes either physically or chemically in blood plasma, then those drugs would not reach its target fully. **Fig. 4** showed that the sample was unstable even from the injection time. Moreover, there were some other peaks showed up in chromatogram which were not TcO_4 and TcO_2.

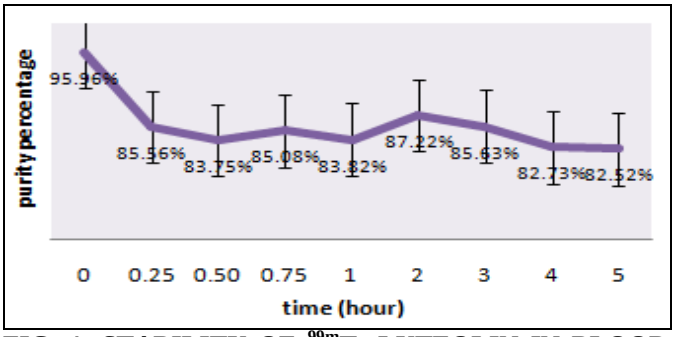

FIG. 4: STABILITY OF ^{99m}Tc-LUTEOLIN IN BLOOD PLASMA

The unstable result of ^{99m}Tc-Luteolin could be affected by the components of blood plasma, such as water, a major component that disturbed the stability of dissolved Luteolin in DMSO [14], the protein that decreased the affinity of Luteolin to technetium [15], and pH difference between optimum pH for the preparation and blood plasma [16].

Lipophilicity Test Result: Lipophilicity (Log P) is a property used to predict a drug's permeability in cell membrane or organs. It also defines the

polarity of the drug to predict its pharmacokinetic and pharmacodynamic properties in the body [17]. Log P was the value of the log partition coefficient of substance in two different polarities - organic solvent and the polar solvent.

In this research, the mean lipophilicity reached 0.95 ± 0.07. This value regarded more lipophilic property. This value was acceptable according to Lipinski Rules of 5 (Ro5)[18] which its lipophilicity was below 5 so that this radiopharmaceutical can be absorbed and well penetrated the cell. Procedurally, the washing steps had been done in this research by a polar solution (which was NaCl 0.9%) to the nonpolar fraction (n-octanol). The two then stabilized in polar and nonpolar phase so that it could minimize bias in the research affected from the uncompleted partition [19].

Protein Plasma Binding Result: Protein plasma binding defined the ability of radiopharmaceutical preparation on binding with blood plasma protein. The binding drugs would be transported to the target organ through the blood circulation. After the target was reached, the free (unbounded) drug would bind to its receptors and gave its pharmacological effects, then continued to be metabolized and got eliminated [20]. Protein plasma binding defined the pharmacokinetic and pharmacodynamic profile of drugs. Although drug should be bounded to reached its target and got to the receptor, too high of protein plasma binding value will also bring disadvantage. The higher the value, the longer time for radiopharmaceutical accumulated in the body and would be difficult to be metabolized and excreted. Those would lead to adverse effect reaction or worse, toxicity [20]. It was found that the protein plasma binding value of ^{99m}Tc-Luteolin was 38.01 ± 4.28%. This would affect drug transportation and blood accumulation which affected the therapeutic mechanism of ^{99m}Tc-Luteolin.

Electric Charge Test Result: Electrical charge test was done by a paper electrophoresis method. Electrophoresis occurred when electrons migrate from anode to cathode so that the cathode would be negatively charged and anode would be positively charged. These two electrodes were separated into two chambers filled with phosphate buffer. The buffer acted as the medium that facilitated migration and stabilizes the medium pH so that it wouldn't disrupt reduction strength of $SnCl_2$ which could disrupt ^{99m}Tc-Luteolin stability [20]. Charges separation of substances were affected by the different migration rate according to its size and amount of charges it contains. It is detected that ^{99m}Tc-Luteolin had no charges (neutral) which were shown by no migration from its original space **Fig. 5**.

Theoretically, if there were a negatively charged radiopharmaceutical, then the migration would likely go to the anode, and the opposite for cathode. Theoretically, if there were a negatively charged substance, the migration would occur from cathode to anode and the opposite for positively charged. When there's no free anion or cation in the preparation, then there would be no electron migration in the chamber [20]. Meanwhile, ^{99m}Tc migrated from cathode to anode. This showed that negative charges or anion presented in the isotope. This result was appropriate to the compound which had free electrons and was negatively charged, TcO_4^-.

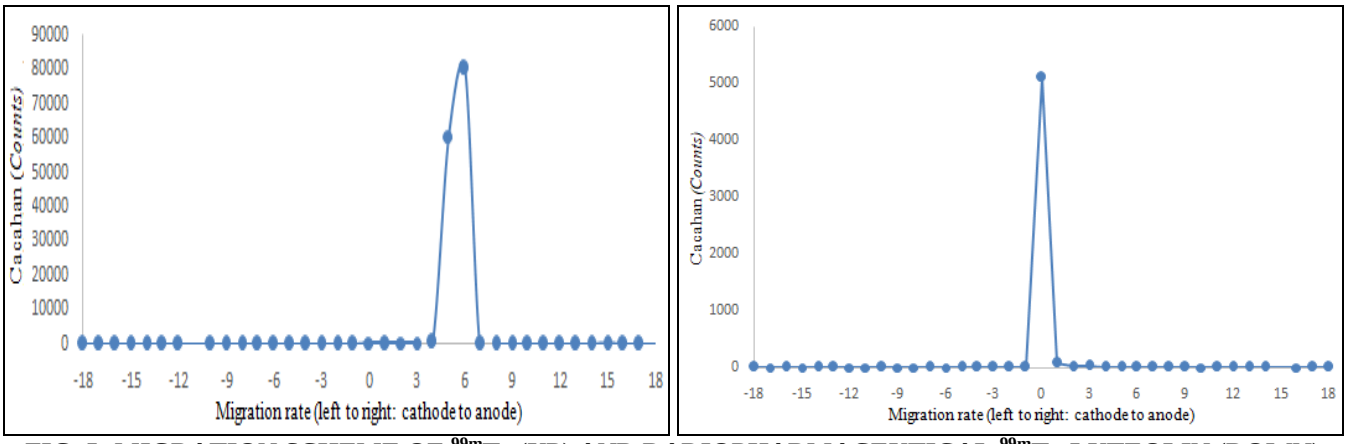

FIG. 5: MIGRATION SCHEME OF ^{99m}Tc (UP) AND RADIOPHARMACEUTICAL ^{99m}Tc-LUTEOLIN (DOWN)

It should be added that to obtain a radiopharmaceutical with high purity values, an optimization activity was needed. Optimization was carried out on several parameters including pH, a number of reducing agents added, and incubation time performed [20]. As a reducing agent, the amount of $SnCl_2$ added must be sufficient so that the reaction can run correctly. This amount will affect the amount of impurity that will change the purity of TcO_4 and TcO_2. Too little amount of $SnCl_2$ cannot reduce $^{99m}Tc7^+$ well so that the amount of TcO_4 impurity will be high. While the amount of $SnCl_2$ that is too much will produce many reduced forms, namely $^{99m}Tc_4^+$ so that the amount of TcO_2 impurity increases [19]. The second factor that affects the purity of the radiopharmaceutical is pH. The pH used can change the reducing effect of $SnCl_2$. Inappropriate pH will then change the amount of impurity, either TcO_4 or TcO_2, which will then affect radiochemical purity [19].

An overly alkaline pH value will eliminate the reduced effect of $SnCl_2$ so that there are lots of TcO_4 (irreducible form) and cannot complex with luteolin. A pH value that is too alkaline will hydrolyze $SnCl_2$ into stannum hydroxide $Sn(OH)_2$ and increase the amount of impurity TcO_4. Whereas the pH that is too acidic will increase the reducing effect of $SnCl_2$ so that more TcO_2 impurities will be formed. Also, the colloidal form of $SnCl_2$ which binds to TcO_2 can affect the physical form of the preparation and reduce the purity of the radiopharmaceutical [21]. The amount of $SnCl_2$ reducing agent and vacuum conditions is very necessary to produce high purity.

Important vacuum to be used to remove air from the container/vial. The air in the vial will slowly oxidize $SnCl_2$ and then reduce its reduction power. On the other hand, the amount of $SnCl_2$ added will also affect the amount of reduced and impurity. If the amount of $SnCl_2$ used is too little, TcO_4 will increase. Conversely, too much amount will increase the amount of $SnO(OH)_2$ which cannot form complex compounds [22]. Both types of impurities can be detected by chromatographic methods, using thin layer chromatography (TLC) plates. The TLC used is KLT SGF254 and ITLC-SG. The mobile phase used is physiological NaCl and C1 (ethanol: water: ammonia = 2: 5: 1) sequentially.

Physiological NaCl can separate TcO_4 with compounds that have been successfully marked, in this case, ^{99m}Tc-Luteolin, with a value of $R_f = 0$ [23]. That is, the desired luteolin compound will remain in the starting point of the bottling and not go up to the tip of the plate like a TcO_4 impurity. The value of TcO_4 alone must be determined to determine the number of impurities that reduce the purity of the radiopharmaceutical. The amount of impurity rising to the end of the plate is calculated by a single channel analyzer (SCA) instrument or a thin layer chromatography scanner (TLC Scanner). While the amount of TcO_2 impurity can be known by eluting the plate with a mixed solvent C1. C1 can separate ^{99m}Tc-Luteolin with TcO_2 with $R_f = 1$. That is, ^{99m}Tc-Luteolin will go up with eluent towards the end of the plate and leave the TcO_2 impurity at the point of bottling [12]. As with TcO_4, impurity values can be calculated using a single channel analyzer (SCA) instrument or a thin layer chromatography scanner (TLC Scanner).

To get a complete purity value, it takes 2 types of plates and 2 different types of eluents. This is because the use of just one is not right to ensure the truth of purity obtained. If you only use eluent C1, then the pure value of ^{99m}Tc-Luteolin will be in the form of accumulation with the TcO_4 impurity which also rises to the end of the plate. While the use of physiological NaCl will refract ^{99m}Tc-Luteolin purity because the accumulation of TcO_2 also occurs at the point of bottling. Both impurity values are needed to ensure and correct the values obtained to obtain true purity [12].

Unlike the KLT plate in general, for radiopharmaceutical testing, the TLC plate used is cut 1 × 1 cm each and given a numbering to facilitate the calculation of the radiation value. This method helps ensure the value and location of ^{99m}Tc-Luteolin radiation. The TcO_2 impurity will reveal a certain amount of radiation on the plate numbered "0" on SGF254 TLC paper while TcO_4 will show a number of radiation numbers "7" and "8" on ITLC-SG paper. This cut will distinguish the amount of radiation in each number and indicate the ^{99m}Tc-Luteolin position. Luteolin with yellow compounds also facilitates the identification of purity of ^{99m}Tc-Luteolin. If at ITLC-SG on a plate number "8" there is a yellow dot and emits radiation that is high during enumeration, then the

radiation can be ascertained to be from ^{99m}Tc-Luteolin, not just $^{99m}TcO_4$ impurity. Likewise, with the TLC-SGF254 plate, the yellow dot on the number plate "0" with high radiation during the enumeration indicates the origin of the radiation is ^{99m}Tc-Luteolin, not TcO_2 only.

A radiopharmaceutical must be used as efficiently as possible so as not to cause dangerous side effects. Radiopharmaceuticals must accumulate in the target organ of the desired body with an adequate amount so that it can be detected during imaging. A radiopharmaceutical that does not achieve purity will certainly affect its effect on the body. Radiopharmaceuticals that do not meet the requirements of purity levels will affect their effects on the body, for example, such as accumulation of radioisotopes in non-target organs [25]. If the accumulation occurs on the wrong target, the imaging done cannot fulfill the initial goal, as in this case, it detects oxidative stress diseases in the body.

Impure radiopharmaceuticals also mean that the marked compounds do not meet the specified amount so that the accumulation of the target organ decreases and affects the imaging in the body. Therefore, radiochemical purity is one aspect of testing the physicochemical characteristics of a radiopharmaceutical in ensuring its safety when used in the body.

From the test results, the radiopharmaceutical purity value was 98.19% ± 1.62%. These results indicated a pure value and qualified as a radiopharmaceutical according to USP which is more than 90%. These results indicated that the formulation of the radiopharmaceutical was optimal, and it was predicted that it could reach the target organ well so that it could emit photons to be detected on gamma cameras.

CONCLUSION: It was found that the physicochemical characteristics of ^{99m}Tc-Luteolin radiopharmaceutical were as follows; radio-chemical purity 98.19% ± 1.62%, storage stability 2 h after incubation and unstable in blood plasma, lipophilic, protein plasma binding 38.01% ± 4.28%, and had no charges (neutral). This result suggests that ^{99m}Tc-Luteolin may be used in distribution *in-vivo* study of luteolin.

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CONFLICT OF INTEREST: All authors have none to declare.

REFERENCES:

1. Lobo V, Patil A, Phatak A and Chandra N: Free radicals, antioxidants and functional foods: Impact on human health, Pharmacogn Rev 2010; 4(8): 118-26.
2. Rahman K: Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging 2007; 2(2): 219-36.
3. Lin Y, Shi R, Wang X and Shen HM: Luteolin, a flavonoid with potentials for cancer prevention and therapy. Curr Cancer Drug Targets 2008; 8(7): 634-46.
4. British Pharmacopeia Comission. British Pharmacopoeia. London: Stationary Office 2013
5. Zainuddin N and Sriyani ME: Characteristics of ^{99m}Tc-Glutathione Radiopharmaceuticals. Journal Sains dan Teknologi Nuklir Indonesia 2012; 13(1): 1-12.
6. Owunwanne A, Patel M and Sadek S: The Handbook of Radiopharmaceuticals. London: Chapman & Hall Medical 2012.
7. Saha GB: Characteristics of Specific Radio-pharmaceuticals, in Fundamentals of Nuclear Pharmacy. New York: Springer 2010: 115-52.
8. Wu CC, Fang CY, Hsu HY, Chuang HY, Cheng YJ and Chen YJ: EBV reactivation as a target of luteolin to repress NPC tumorigenesis'. Oncotarget 2016; 7(14): 18999-017.
9. Khoramrouz SJ, Erfani M and Allaf MA: Technetium-^{99m} tricarbonyl labeled a broad-spectrum quinolone as a specific imaging agent in infectious diseases. Iranian Journal of Pharmaceutical Research 2017; 16(2): 611-18.
10. Zainuddin N, Sriyani ME and Widyasari EM: Development of ciprofloxacin radiopharmaceutical formulations in a single container, Majalah Farmasi Indonesia 2010; 21(2): 139-49.
11. Nurlaila Z, Maula ES and Eva MW: Development of ciprofloxacin radiopharmaceutical formulation in a single vial. 2010. Available from: http://i-lib.ugm.ac.id/jurnal/detail.php?dataId=12134 (Accessed on October 1, 2018).
12. Lowe GD, Rumley A and Mackie I: Plasma Fibrinogen. Annals of Clinical Biochemistry 2004; 4(6): 430-440
13. Gandomkar M, Najafi R, Mazidi M, Mirfallah SH and Goudarzi M: Three different procedures in labeling of Ubiquicidin with technetium 99m: a comparative study. Iran J Radiat Res 2009; 7: 97-104.
14. Xu L, Li L, Huang J, Yu S, Wang J and Li N: Determination of the lipophilicity (log Po/w) of organic compounds by microemulsion liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis 2015; 102: 409-16.
15. Bhardwaj N, Bhatnagar A and Singh AK: Development and evaluation of a single vial cold kit for infection imaging: Tc-99m ciprofloxacin. World J Nucl Med 2005; 4: 244-51.
16. Gandomkar M, Najafi R, Shafiei M, Mazidi M, Goudarzi M, Mirfallah SH, Ebrahimi F, Heydarpor HR and Abdie N: Clinical evaluation of antimicrobial peptide [(99m)Tc/Tricine/HYNIC(0)]ubiquicidin 29- 41 as a human-specific infection imaging agent. Nucl Med Biol 2009; 36: 199-05.

17. Sakai JB: Pharmacokinetics: The absorption, distribution, and excretion of drugs, in practical pharmacology for the pharmacy technician. Canada Wolters Kluwer 2008: 27-40.
18. Pál G: Electrophoresis, in Introduction to Practical Biochemistry. Hungaria: ELTE Faculty of Natural Sciences, Institute of Biology 2013.
19. Misyetti M and Daruwati I: Labelling CTMP with Technetium-^{99m} for Bone Cancer Investigators. Jurnal Sains dan Teknologi Nuklir Indonesia 2008; 9(1): 79-88.
20. Zolle I: Technetium-^{99m} pharmaceuticals: preparation and quality control in nuclear medicine. Berlin: Spr 2007: 7-66
21. Erfani M, Doroudi A, Hadisi L, Andishmand A, Mirshojaei SF and Shafiei M: 99mTc-tricarbonyl labeling of ofloxacin and its biological evaluation in *Staphylococcus aureus* as an infection imaging agent. J. Label. Compd. Radiopharm 2013; 56: 627-31.
22. Nurlaila Z and Sriyani M: Radiopharmaceutical characteristics of ^{99m}Tc-Glutathione. Jurnal Sains dan Teknologi Nuklir Indonesia 2012; 13(1): 1-12.

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