ORIGINAL ARTICLE

Technetium-99m-labeled genistein as a potential radical scavenging agent

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ABSTRACT

The purpose of this study was to determine the optimum conditions for labeling genistein compounds with technetium-99m (^{99m}Tc) radionuclides and the percentage of purity obtained in accordance with the requirements of the United State Pharmacopeia. The method used is optimization of several parameters including pH, $SnCl_2.2H_2O$ as reducing agents, genistein concentration, and incubation time. The results showed that the optimum conditions for labeling ^{99m}Tc-Genistein were obtained under conditions of pH 8, the amount of $SnCl_2.2H_2O$ reducing agents was 30 μg, 0.5 mg genistein, and in 10 min. The optimization of this condition resulted in radiochemical purity in the labeling of ^{99m}Tc-Genistein compounds at 95.43% ± 0.85%. The radiochemical purity of the labeling of ^{99m}Tc-Genistein compounds has met the requirements of the United State Pharmacopeia as a compound marked for diagnosis of more than 90%.

Key words: Free radicals, genistein, radiochemical purity, radiopharmaceutical, technetium-99m-genistein, technetium-99m

INTRODUCTION

Cancer is the main disease that causes the biggest death in the world and caused deaths of around 8.2 million in 2012. The biggest cause of cancer deaths every year is caused by lung cancer, breast cancer, liver cancer, stomach cancer, and colorectal cancer.[1]

In Indonesia, about 65% of people with breast cancer come to the doctor at an advanced stage; this indicates the patient is late to detect breast cancer.[2]

Radiopharmaceuticals are compounds containing radioactive elements used for diagnostic purposes and therapeutic treatments for human diseases. Radiopharmaceuticals currently used are more than 95% more for diagnostic purposes and about 5% are used for therapeutic treatment. The application of the use of a compound labeled Technetium-99m (^{99m}Tc) in nuclear medicine is very dominant, which is more than 80%.[3]

^{99m}Tc for diagnostic purposes because it can emit pure gamma rays (140.5 keV) and has a short half-life time of around 6 h. Short half-life time is expected how the radiation emitted by ^{99m}Tc can be used up immediately after the diagnosis process is complete so that the impact of radionuclide exposure can be minimized.[4]

The main requirement of radionuclides and ligands for the diagnosis of cancer is that they can bind to the receptors to form complex compounds. One of the ligands that can be used is genistein. Genistein is an isoflavone compound that has pharmacological effects as anticancer and is able to bind to estrogen receptors

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with the nature of the selective estrogen receptor modulators.[5]

MATERIALS AND METHODS

Paper chromatography, dose calibrator (Victoreen®), 5 µL micropipette, 10–100 µL, and 100–1000 µL (Eppendorf®), analytic balance (Mettler Toledo® Type AL 204), oven (Memmert®), Single Channel Analyzer (SCA) (ORTEC®), syringe (Terumo®), 10 mL glass vial.

The materials used are genistein (Sigma Aldrich®), acetone (Merck®), aquabidestilata (IKA Pharma®), DMSO, HCl 0.1 N, Na $^{99m}TcO_4^-$ (PT. Ansto), Physiological NaCl (IKA Pharma®), NaOH 0, 1 N, universal pH indicator (Merck®), KLT SGF-254 (Merck®) plate, instant thin layer chromatography-silica gel (ITLC-SG) (Agilent Technologies®), and $SnCl_2 \cdot 2H_2O$ plates (Sigma Aldrich®).

Optimization of pH

The determination of the optimum pH used in labeling genistein with $^{99m}TcO_4^-$ used is pH 3; 4; 5; 6; 7; 7.5; 8; 9; and 10. Stock genistein solution is added with a solution of $SnCl_2 \cdot 2H_2O$, and the pH is adjusted by adding NaOH or HCl. After pH is obtained, then a $^{99m}TcO_4^-$ solution is added. The solution was formed, then it was dropped on the KLT SGF-254 and ITLC-SG plates to determine the purity of the $^{99m}TcO_4^-$ Genistein complex.[6]

Optimization of concentration $SnCl_2.2H_2O$ solution

The determination of the optimum concentrations of $SnCl_2.2H_2O$ solution was used five variations in the concentration of $SnCl_2.H_2O$: 10, 20, 30, 40, and 50 µL. After adding 100 µL of genistein solution, each solution is adjusted to optimum pH. After that, each solution was added with a solution of $^{99m}TcO_4^-$ as much as 500 µL, then, the volume of the vial was equated with the addition of NaCl physiological solution to 2 mL and incubated for 30 min. The solution was formed, then dropped on the KLT SGF-254 and ITLC-SG plates to determine the purity of the ^{99m}Tc-Genistein complex.[6]

Optimization of concentration genistein

The determination of optimum genistein concentrations used nine variations in genistein: 1; 2; 3; 4; 5; 6; 7; 7.5; and 10 mg/mL. Each solution was added with a solution of $SnCl_2.2H_2O$, and also added $^{99m}TcO_4^-$ as much as 500 µL. After that, NaCl physiological solution was added to 2 mL and incubated for 30 min. Checking the purity of ^{99m}Tc-Genistein complexes was done by dripping each solution on the KLT SGF-254 and ITLC-SG plates.

Optimization of incubation time

Determination of the optimum incubation time on marking genistein with $^{99m}TcO_4^-$ used five variations of incubation time, namely 5, 10, 15, 30, and 45 min. Each solution of genistein added a solution of $SnCl_2.2H_2O$ in accordance with the optimum results. The pH conditions are adjusted according to the optimum pH that has been obtained. After that, each solution was added with a solution of $^{99m}TcO_4^-$ and NaCl physiological solution. Checking the purity of ^{99m}Tc-Genistein complexes was done by dripping each solution on the KLT SGF-254 and ITLC-SG plates.[6]

The purity percentage of ^{99m}Tc-genistein compounds

The purity of the compound marked ^{99m}Tc-Genistein was determined using the TLC method which was then analyzed using SCA. The stationary phase used is the KLT SGF-254 and ITLC-SG plates. For the mobile phase, two solvents are used, namely C_1 solution consisting of ethanol: water: ammonia (2: 5: 1) and NaCl physiological solution.[7]

The purity percentage of a compound labeled ^{99m}Tc-Genistein is calculated based on the percentage of $^{99m}TcO_4^-$ and $^{99m}TcO_2$ (impurity) using the following equation.[8]

$$\%\ ^{99m}TcO_2\ (\text{reduced}) = \frac{99\ mTc - SnCl2.2H_2O}{\text{total number of counts}} \times 100\%$$

$$\%\ ^{99m}TcO4^- = \frac{^{99m}TcO4}{\text{total number of counts}} \times 100\%$$

Calculation of labeled compounds ^{99m}Tc-Genistein:

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

RESULTS

Testing of optimum pH conditions

The pH factor is important in the stability of compounds marked and can affect the reduction power of the reducing agent used, namely $SnCl_2.2H_2O$.[5] The results for pH optimization testing and radiochemical purity of ^{99m}Tc-genistein labeled compounds are shown in Table 1 and Figure 1.

Test for optimum concentration of $SnCl_2.2H_2O$

$SnCl_2$ is reducing agent when in the form of Sn^{2+} Sn^{2+} is often used to reduce the level of reduction in medium with a low level of toxicity.[9] The results for the optimization of $SnCl_2.2H_2O$ solutions and radiochemical purity of 99mTcgenistein labeled compounds are shown in Table 2 and Figure 2.

Optimum concentration of genistein

Determination of the third parameter is the concentration of genistein solution, the concentration used is 1; 2; 3; 4; 5; 6; 7; 7.5; and 10 mg/mL. Ligands used to make radiopharmaceutical kits, should have a purity of 99% for other marked compounds derived from ligand impurities.[9,10] The result for optimization concentration of genestein and radiochemical purity of ^{99m}Tc-genistein labeled compounds are shown in Table 3 and Figure 3.

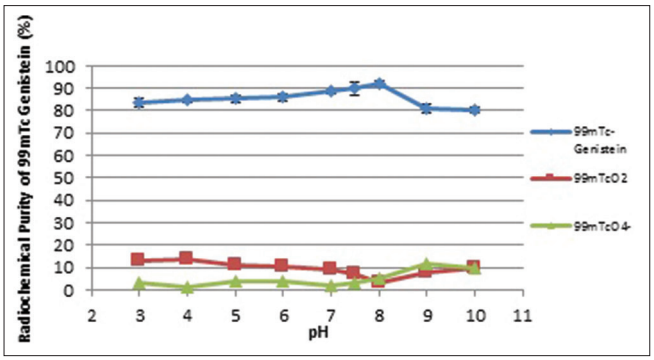

Figure 1: The optimum pH and radiochemical purity of ^{99m}Tc genistein labeled compounds

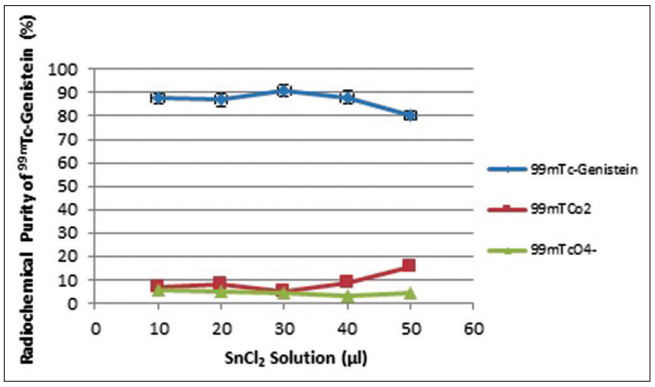

Figure 2: The optimum $SnCl_2$ solution and radiochemical purity of ^{99m}Tc genistein labeled compounds

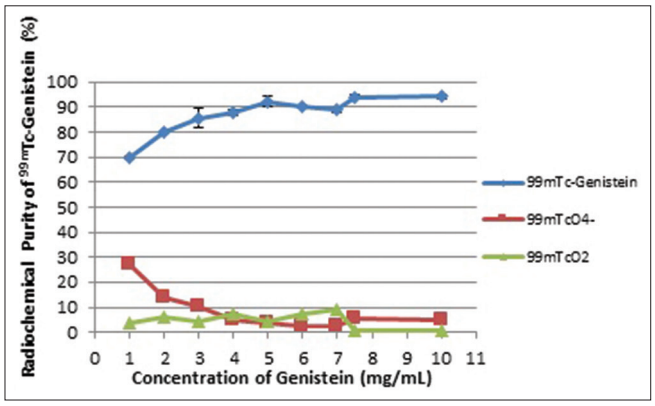

Figure 3: The optimum concentration of genestein and radiochemical purity of ^{99m}Tc genistein labeled compounds

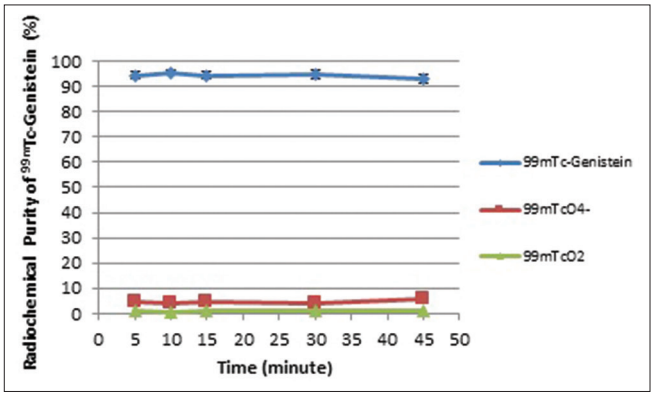

Figure 4: The optimum incubation time and radiochemical purity of ^{99m}Tc genistein labeled compounds

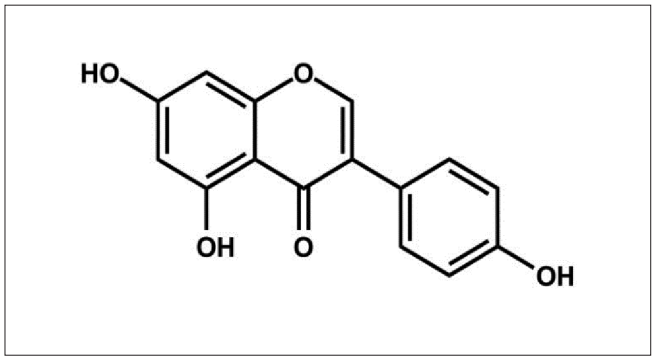

Figure 5: Structure of genestein

The ideal incubation condition is at room temperature because to facilitate the preparation of compounds marked. The incubation time variations carried out were 5, 10, 15, 30, and 45 min. Variation in incubation time is needed to determine the optimum time for Sn^{2+} to reduce $^{99m}TcO_4^-$ to $^{99m}TcO_2$. The result for optimization of incubation time and radiochemical purity of ^{99m}Tc-genistein labeled compounds are shown in Table 4 and Figure 4.

DISCUSSIONS

^{99m}Tc radionuclides can form complex compounds with other elements into radiopharmaceutical preparations. The radiopharmaceutical preparation can be used for diagnosis. After being given to patients, radiopharmaceutical preparations can localize certain organs so that imaging can be carried out in the body based on cell function and physiology.[4] Tc can bind to the ligand to form a complex bond that has an electron donor group. The structure of genestein can be seen in Figure 5.[6]

The purity test of a ^{99m}Tc-Genistein compound can be analyzed by the TLC method. The stationary phase used is thin-layer chromatography TLC SG F254 with the mobile phase of physiological NaCl solution to separate the form of $^{99m}TcO_4^-$, $^{99m}TcO_4^-$ impurity will move toward the peak while ^{99m}Tc-Genistein will remain at the bottling point and ITLC-SG stationary phase with C_1 mobile phase to separate $^{99m}TcO_2$. The $^{99m}TcO_2$ impurity will remain at the bottling point, and ^{99m}Tc-Genistein will move towards the peak.[11]

The results at pH 8 showed that the optimum purity of ^{99m}Tc-Genistein was 91.97% ± 1.43% with impurity of $^{99m}TcO_2$ 3.05% ± 0.45% and impurity $^{99m}TcO_4^-$ 4.98% ± 1.16%. At a pH which is more basic than 8, it produces a higher

Table 1: The Optimum pH and Radiochemical Purity of ^{99m}Tc Genistein Labeled Compounds

pH	Average Radiochemical Purity (%)			Description
	Polluter $^{99m}TcO_2$	Polluter $^{99m}TcO_4^-$	^{99m}Tc-Genistein	
3	13.23±0.46	3.38±1.74	83.40±2.08	cloudy
4	13.88±0.77	1.46±0.07	84.66±0.89	cloudy
5	10.77±0.66	3.83±1.18	85.40±1.56	cloudy
6	10.45±1.20	3.59±0.78	85.96±1.65	cloudy
7	9.28±0.58	2.09±0.79	88.64±1.13	cloudy
7,5	7.19±1.94	2.98±1.73	89.83±3.01	cloudy
8	3.05±0.45	4.98±1.16	91.97±1.43	clear, yellowish
9	7.59±0.96	11.47±1.16	80.94±1.74	clear, yellowish
10	9.72±0.40	9.90±1.15	80.38±1.40	clear, yellowish

Table 2: The Optimum Concentration of SnCl2.2H2O and Radiochemical Purity of ^{99m}Tc genistein labeled compounds

$SnCl_2 \cdot 2H_2O$ solution	Average Radiochemical Purity (%)			Description
	Polluter $^{99m}TcO_2$	Polluter $^{99m}TcO_4^-$	^{99m}Tc-Genistein	
10 µl	7.02±1.15	5.36±1.37	87.62±2.06	clear, yellowish
20 µl	8.08±1.71	5.05±1.74	86.87±2.82	clear, yellowish
30 µl	5.06±1.13	4.10±0.94	90.84±2.38	clear, yellowish
40 µl	8.76±2.11	3.25±0.94	87.99±2.67	clear, yellowish
50 µl	15.51±1.63	4.26±0.69	80.22±2.05	clear, yellowish

Table 3: The Optimum Concentration of Genistein Solution and Radiochemical Purity of ^{99m}Tc-Genistein Labeled compounds

Concentration of Genestein	Average Radiochemical Purity (%)			Description
	Polluter $^{99m}TcO_2$	Polluter $^{99m}TcO_4^-$	^{99m}Tc-Genistein	
1 mg/mL	3.49±0.39	27.00±0.37	69.51±0.62	clear, yellowish
2 mg/mL	6.28±0.19	13.99±0.05	79.73±0.23	clear, yellowish
3 mg/mL	4.39±2.52	10.13±2.03	85.48±3.74	clear, yellowish
4 mg/mL	7.35±0.58	4.71±0.91	87.94±1.25	clear, yellowish
5 mg/mL	4.07±1.28	3.74±0.97	92.19±1.86	clear, yellowish
6 mg/mL	7.39±0.58	2.67±0.25	89.94±0.73	cloudy
7 mg/mL	8.86±0.16	2.36±0.75	88.78±0.88	cloudy
7.5 mg/mL	0.73±0.52	5.34±0.72	93.92±1.03	cloudy
10 mg/mL	0.83±0.70	5.15±0.70	94.02±1.15	cloudy

Table 4: The Optimum Incubation Time of Genistein Solution and Radiochemical Purity of ^{99m}Tc-Genistein Labeled Compounds

Incubation time (min)	Average Radiochemical Purity (%)			Organoleptis
	Polluter $^{99m}TcO_2$	Polluter $^{99m}TcO_4^-$	^{99m}Tc-Genistein	
5	1.30±0.71	4.39±1.17	94.31±1.58	clear, yellowish
10	0.57±0.14	4.00±0.72	95.43±0.85	clear, yellowish
15	0.94±0.41	4.90±1.14	94.16±1.39	clear, yellowish
30	1.08±0.45	4.34±1.34	94.58±1.64	clear, yellowish
45	1.22±0.42	5.88±1.39	92.90±1.67	clear, yellowish

$^{99m}TcO_4^-$ impurity, because at a higher pH, Sn (II) will be hydrolyzed to stano hydroxide which is no longer functioning as a reducing agent. Whereas in more acidic pH conditions, it will increase the amount of impurity $^{99m}TcO_2$ because the reducing agent $SnCl_2.2H_2O$ will reduce more strongly in acidic conditions.[9,10,12]

The results obtained on the optimum number of $SnCl_2.2H_2O$ solutions were 30 µl with a purity of 90.84% ± 2.38% with impurities of $^{99m}TcO_2$ 5.06% ± 1.13% and impurities $^{99m}TcO_4^-$ 4.10% ± 0.94%. If the amount of the solution of $SnCl_2.2H_2O$ is more partial, $SnCl_2$ is hydrolyzed to form its hydroxide which will then bind to ^{99m}Tc-reduced to form

$^{99m}TcO_2$ colloid, so that the amount of impurity will be more $^{99m}TcO_2$.[13]

The optimization of genistein concentration was obtained at 10 mg/mL, but the solution produced was cloudy, as well as at 6, 7, and 7.5 mg/mL. Requirements for intravenous injection preparation, the solution produced must be clear. Therefore, the optimum concentration used was a concentration of 5 mg/mL, with a purity of 92.19% ± 1.86%.

The incubation time is strongly related to the preparation of a radiopharmaceutical preparation to be used. This is related to the effectiveness of the half-life of radionuclide used, and the purpose of therapy or diagnosis. The incubation time obtained is ideal for the application of 10 min. In the next minute, there was a relatively low decrease in purity and was not significant. The optimization of incubation time of ^{99m}Tc-Genistein was obtained 10 min with optimum purity of ^{99m}Tc-Genistein, which was 95.43% ± 0.85% with impurity of $^{99m}TcO_2$ 0.57% ± 0.14% and impurity $^{99m}TcO_4^-$ 4.00% ± 0.72%.

CONCLUSIONS

The optimum condition for marking ^{99m}Tc-Genistein produced radiochemical purity of 95.43% ± 0.85%. This fulfills the radiochemical purity requirements set by the United State Pharmacopeia as marked compounds for diagnosis of more than 90%.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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