WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 1, 1865-1871. Research Article ISSN 2277– 7105

OPTIMIZATION OF APIGENIN LIGAND IN THE ^{99M}TC-APIGENIN COMPOUND SYNTHESIS PROCESS AS A POTENTIAL RADICAL SCAVENGER AGENT

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Article Received on
15 Nov. 2019,

Revised on 06 Dec 2019,
Accepted on 27 Dec. 2019

DOI: 10.20959/wjpr20201-16600

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ABSTRACT

Objective. The purpose of this study is to find the optimum conditions of apigenin ligands in the ^{99m}Tc-Apigenin compound synthesis process. The antioxidant activity of apigenin can be used to detect the presence of excess free radicals in the body. The compound formed is expected to be a radiotracer compound for cancer diagnosis. **Methods.** The method used in the synthesis of ^{99m}Tc-Apigenin compounds is the amount of apigenin ligands. The optimal concentration of apigenin ligand can be evaluated from the acquisition value of the best radiochemical purity. **Results.** The results of optimization of the number of Apigenin ligands in the synthesis of ^{99m}Tc-Apigenin compounds obtained optimal apigenin concentration was 0.5 mg with a radiochemical purity value of 85,93%±1,10%. **Conclusion.** The optimal amount of apigenin ligand is 0.5 mg in the synthesis reaction of 99mTc-Apigenin compound, with the least amount of $^{99m}TcO_4^-$ and TcO_2 impurities at 4.14%±0.03% and 9.94%±1.12%.

KEYWORDS: Apigenin, ligand, technetium-99m, radiochemical purity, ^{99m}Tc-apigenin, radiotracer.

INTRODUCTIONS

Conditions of oxidative stress in the body are caused by overproduction of the amount of reactive oxygen (ROS), which can oxidize lipids, proteins and DNA, and cause damage to

cell membranes, and produce several diseases. Atherosclerosis, heart disease, stroke, vascular disease, diabetes, cancer and premature aging can be caused by conditions of oxidative stress.[1,2] This condition can be caused by several other factors, such as unhealthy lifestyles, stress, drinking alcohol, smoking, pollution, consuming fast food, and improper dietary habits.[3]

The reactivity of these high ROS concentrations can disrupt the balance of antioxidant and prooxidant levels.[4] Research has proven that natural compounds such as flavonoids are needed that can reduce oxidative stress and improve immune function.[5] Apigenin compounds have the chemical structure of flavones that are non-toxic and non-mutagenic, are widely distributed in many fruits and vegetables such as parsley, onions, orange, tea, chamomile, and wheat sprouts and in some seasons.[6] Apigenin has diverse biological effects, including improvement of the cancer cell response to chemotherapy, tumorigenesis, modulating immune cell function, and anti-platelet activity.[7,8,9]

In the field of nuclear medicine, as a producer of gamma rays, technetium-99m is widely used for the purposes of diagnosing cancer or metabolic disorders.[10] This labeled structure can be used to obtain images with a single photon computerized tomography (SPECT) emission showing physiological status from a network.[11] ^{99m}Tc, as sodium pertechnetate, has also been used to label structures to observe biological activity.[12] The use of radionuclides in nuclear medicine due to Technetium-99m has optimal physical and chemical characteristics (half-life and biological physics, low gamma energy emissions of 140 keV, availability of 99Mo/^{99m}Tc generators and negligible environmental impacts).[13]

MATERIALS AND METHODS

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

The materials used are apigenin(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 $SnCl_2.2H_2O$ (Sigma Aldrich®).

Optimization of ligand Apigenin

Determination of the optimization of the number of apigenin ligands in the synthesis of ^{99m}Tc-Apigenin compounds, used 5 variations of the amount of apigenin 300, 400, 500, 600, and 700 µL. Five vials measuring 10 mL were marked (A, B, C, D, and E), then each vial was inserted the variation of the number apigenin. After that, each vial was added with a $SnCl_2.2H_2O$ reducing agent. After that, each vial is added with a solution of Sodium Hydroxide or Hydrochloric Acid to obtain pH 6 as the optimum pH. The five vials were then added with a solution of $^{99m}TcO_4^-$ 300 mL and incubated for 30 minutes. Evaluation of the purity of the ^{99m}Tc-apigenin complex compound formed was determined by the radiochemical purity measured by measurements on the GF-254 Silica gel TLC and ITLC-SG plates.

The Purity Percentage of ^{99m}Tc-Apigenin Compounds

The purity of the compound marked ^{99m}Tc-Apigenin was determined using the thin layer chromatography (TLC) method which was then analyzed using Single Channel Analyzer (SCA). The stationary phase used is the KLT SGF-254 and ITLC-SG plates. For the mobile phase, 2 solvents are used, namely C_1 solution consisting of ethanol: water: ammonia (2: 5: 1) and NaCl physiological solution.[14]

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

$$\%\ 99mTcO2\ (reduced) = \frac{99mTc - SnCl2.2H2O}{\text{total number of counts}}\ x\ 100\%$$

$$\%\ 99mTcO4^- = \frac{99mTcO4}{\text{total number of counts}}\ x\ 100\%$$

Calculation of labeled compounds ^{99m}Tc-Apigenin

$$\%\ ^{99m}\text{Tc-Apigenin} = 100\% - (\%\ ^{99m}TcO_2 + \%\ ^{99m}TcO_4^-).$$[15]

RESULTS AND DISCUSSION

Apigenin bioactive compounds will undergo metabolism and have a pharmacokinetic profile that affects tissue distribution and bioactivity. In nature, apigenin also occurs linked via C-C or C-O-C bonds from the dimeric form. The structure of flavonoids in the form of aglycones and glycosides will affect the pharmacokinetic profile and also different healing results.[16] The results of the study indicate the bioavailability of apigenin-C-glycosides, reported absorption of vitexin-2 Unchanged O-xyloside (VOX), an apigenin-8-C-glucoside in a mouse

model. Apigenin-8-C-glycosides undergo enterohepatic recirculation in addition to hydrolysis of monoglycosides, reductions, and conjugations to form glucuronides that are available biologically.[17]

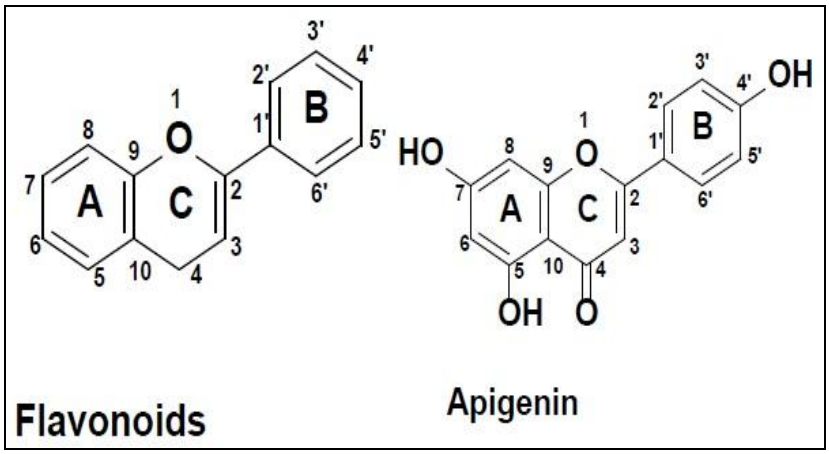

Fig. 1: Basic Structure Flavonoid And Apigenin.

The ^{99m}Tc-Apigenin marked compound is formed from the co-ordinated covalent bond between technetium-99m as a metal core and apigenin as a ligand. apigenin will form complexes with Mg metal in the hydroxyl group on C_5 in ring A and the 4-C = O group in ring C.[18] The reactions that occur in the ^{99m}Tc-Apigenin complexation reaction are as follows:

$$\text{Apigenin} + Sn^{2+} + {}^{99m}Tc^{(VII)}O_4^- \rightarrow {}^{99m}Tc^{(IV)}\text{Apigenin} + Sn^{4+} + {}^{99m}TcO_2 + {}^{99m}TcO4^-$$

From this reaction it is known that in addition to producing ^{99m}Tc-Apigenin labeled compounds, there are also side compounds (radiochemical impurities) in the form of $^{99m}TcO_2$ and $^{99m}TcO_4^-$excess which can affect the purity of compounds labeled ^{99m}Tc-Apigenin.

Optimization results for the number of apigenin (ligand) conditions

Optimization of the amount of apigenin (ligand) in the synthesis of ^{99m}Tc-apigenin compounds is very important in obtaining radiochemical purity of complex compounds formed. In this study 5 variations of the formula for the amount of apigenin (ligand) used are shown in Table 1.

Table 1: Variation of Formulas In The Number of Apigenin (Ligand) Optimization.

Vial	Apigenin (mg/600 µL)	pH	$SnCl_2$ (µL)	HCl 0.1 M (µL)	NaOH 0.1 M (µL)	H_2O (µL)	TcO_4^- (µL)	Incubation Time (min)
A	0.2	6	20	-	20	120	250	30
B	0.3	6	20	-	30	100	250	30
C	0.4	6	20	-	40	80	250	30
D	0.5	6	20	-	50	60	250	30
E	0.6	6	20	-	60	40	250	30

The results of the five formulas in the number of apigenin (ligand) can be seen the average radiochemical purity results shown in Table 2 and Figure 2.

Table 2: Percentage of radiochemical purity of variations in apigenin (ligand).

Apigenin (mg)	0.2	0.3	0.4	0.5	0.6
Radiochemical Purity (%)	71.29±0.5	69.65±1.18	81.07±0.86	85.93±1.10	81.14±0.74
TcO_2^- (%)	25.54±1.21	25.60±1.09	13.53±2.50	9.94±1.12	15.77±2.58
$TcO4^-$ (%)	3.16±1.24	4.75±1.24	5.40±2.70	4.14±0.03	3.09±1.96

Table 2. Percentage of radiochemical purity of variations in apigenin (ligand).

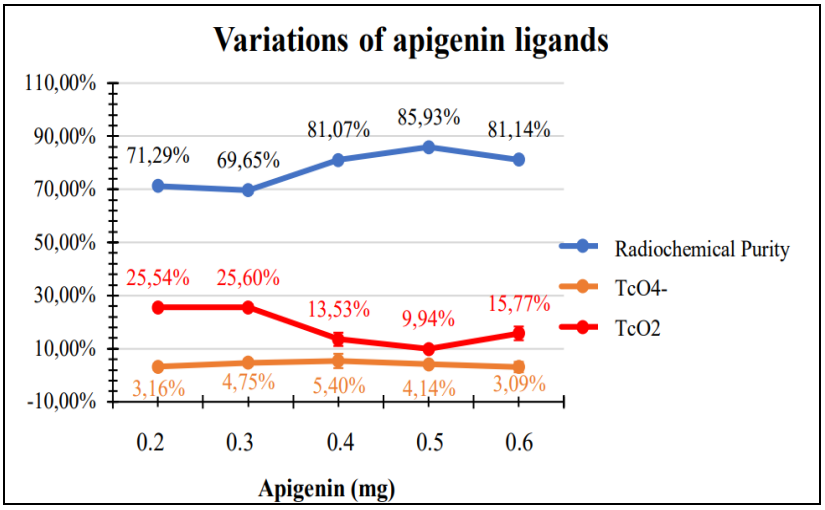

Fig. 2: Radiochemical purity in the number of apigenin (ligand) variations.

The results of optimization of the amount of apigenin (ligand) were evaluated from the highest percentage of purity at the amount of apigenin 0.5 mg with a purity of 85.93% ± 1.10%. The use of apigenin amounts of 0.3 mg and 0.2 mg will reduce the purity percentage of the compound compound marked ^{99m}Tc-Apigenin as the percentage of impurity increases $^{99m}TcO_2$. This is because the ligands in the sample are too small to react with TcO_4^-. The number of ligands will be directly proportional to the amount of TcO_4^- that has been reduced by the $SnCl_2.2H_2O$ reducing agent.

CONCLUSIONS

The result of labeling the flavonoid compound with the teknesium-99m radionuclide obtained the condition of the optimum amount of apigenin ligand 0.5 mg with radiochemical purity of 85.93% ± 1.10%.

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