WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 1, 433-439. Research Article ISSN 2277– 7105

# DETERMINATION OF THE OPTIMUM CONCENTRATION OF $SNCL_2.2H_2O$ AS A REDUCING AGENT IN THE LABELING OF APIGENIN COMPOUNDS WITH RADIONUCLIDE TECHNETIUM-99M AS A RADIOTRACER COMPOUND FOR CANCER DIAGNOSIS

**Danni Ramdhani[1]*, Maula Eka Sriyani[2], Marisa Dwi Ariani[1], Eva Maria Widyasari[2]**

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363.

[2]Applied Nuclear Science and Technology Center (PSTNT), National Atomic Energy Agency (BATAN), Bandung, West Java, Indonesia 40116.

Article Received on
12 Nov. 2019,

Revised on 02 Dec. 2019,
Accepted on 22 Dec. 2019,

DOI: 10.20959/wjpr20201-16571

***Corresponding Author**
**Danni Ramdhani**
Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363.

## ABSTRACT

**Objective:** The purpose of this study is to determine the optimization of the amount of $SnCl_2.2H_2O$ reducing agent in the process of labeling Apigenin flavonoid compounds with Technetium-99m radionuclide compounds. The compound formed is expected to be a radiotracer compound for cancer diagnosis. **Methods:** The method used in optimizing the $^{99m}$Tc-Apigenin compound labeling is the number of $SnCl_2.2H_2O$ reducing agents. The optimum reducing agent concentration can be evaluated from the value of obtaining the best radiochemical purity value. **Results:** The results of optimization of the number of $SnCl_2.2H_2O$ reducing agents on the synthesis of $^{99m}$Tc-Apigenin compound obtained the optimum concentration of $SnCl_2.2H_2O$ is 0.02 mg with a radiochemical purity value of 74.88% ± 1.86%. **Conclusion:** The optimum amount of $SnCl_2.2H_2O$ reducing agent is 0.02 mg for $^{99m}$Tc-Apigenin labeling, with the least amount of $^{99m}TcO_4^-$ and $TcO_2$ impurities at 21.99%±2.02% and 3.13%±0.49%.

**KEYWORDS:** Apigenin, reducting agent, $SnCl_2.2H_2O$, Technetium-99m, Radiochemical Purity, $^{99m}$Tc-Apigenin, Radiotracer.

## INTRODUCTIONS

Flavonoids are the most common and widespread secondary metabolic compounds of the phenolic group, occurring in almost all parts of plants, especially leafy plants which carry out photosynthesis. Flavonoids are the main coloring component of flowering plants. Flavonoids are an integral part of human and animal food.[1] Consumption of foods containing flavonoids plays an important role in cancer prevention. Fruits and vegetables that have flavonoids have been reported as cancer chemopreventive agents.[2,3]

The structure of flavonoids consists of a group of polyphenol compounds which have a benzo-r-pyrone structure and are widely contained in plants. This compound can be synthesized via the phenylpropanoid pathway. The results showed that secondary metabolites of phenolic nature including flavonoids had many pharmacological activities.[4,5]

Flavonoids generally have antioxidant activity. The antioxidant activity of flavonoids depends on the regulation of functional groups on their chemical structure. This relates to the configuration, substitution, and number of hydroxyl groups that are owned.[6] The hydroxyl B ring configuration is the most significant determinant of reducing ROS and RNS because it donates hydrogen and electrons to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and producing relatively stable flavonoid radicals.[7]

In the field of nuclear medicine, as a producer of gamma rays, technetium-99m is widely used label molecular and cellular structures (radiopharmaceutical or radiobiocomplex). This labeled structure can be used to obtain images with a single photon computerized tomography (SPECT) emission showing physiological status from a network.[8] $^{99m}$Tc, as sodium pertechnetate, has also been used to label structures to observe biological activity.[9] 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).[10,11]

## 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 reductor $SnCl_2.2H_2O$**

In determining the optimum conditions the number of $SnCl_2.2H_2O$ reducing agents used 5 variations, namely 10, 20, 30, 40, and 50 µL with concentrations of 1 µg/µL. Five vials of 10 mL were marked (A, B, C, D, and E) given 600 µL of apigenin solution and also $SnCl_2H_2O$ solution of 10, 20, 30, 40, and 50 µL. After that, a solution of Sodium Hydroxide or Hydrochloric Acid is added to each vial to obtain the optimum pH which is pH 6. The addition of aquabidest is added to each vial to obtain a volume of 1 mL / each vial. Then each vial was added with a solution of $^{99m}TcO_4^-$ 300 mL and incubated for 30 minutes. After the incubation process, each solution was dropped on a TLC Silica gel $GF_{254}$ plate to determine the purity of the $^{99m}$Tc-Apigenin complex compound.[12]

**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.[12]

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.

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

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

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

% $^{99m}$Tc-Apigenin = 100% - (% $^{99m}TcO_2$ + % $^{99m}TcO_4^-$).[13]

## RESULTS AND DISCUSSION

Apigenin has a chemical structure as 4, 5, 7, -trihydroxyflavone in the form of yellow crystalline powder including the class of flavone, which is an aglycone of some naturally occurring glycosides. Apigenin has been proven to have pharmacological activity, including anti-inflammatory, anti-toxic, anti-cancer. Apart from that apigenin has also been shown to have many molecular targets involved in inflammation. Based on in vivo, in vitro and clinical trials, apigenin is a powerful therapeutic agent for treating diseases such as rheumatoid arthritis, autoimmune disorders, Parkinson's disease, Alzheimer's disease, and various types of cancer.[14,15]

Technetium-99m is a radionuclide for diagnostic purposes which is widely used in nuclear medicine because it has ideal physicochemical properties. Technetium-99m has its nuclear decay character ($t_{1/2}$= 6 hours, Ec = 140 keV). Technetium is bound to the carrier[16] which sends radionuclides to certain sites in the body for imaging.

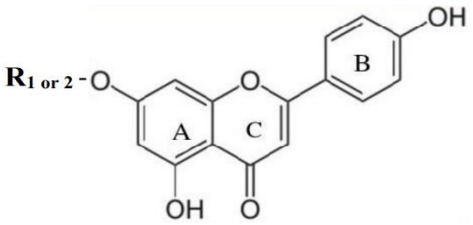


**Fig. 1: Structure of apigenin and its glycosides R1: apiin; R2: apigenin-7-O-glycoside.**

### Optimization results for $SnCl_2.2H_2O$ conditions

Determination of optimization of the number of reductants in the synthesis of compounds marked $^{99m}$Tc-Apigenin aims to reduce 99mTcO4- so that they can bind to ligands. In this study $SnCl_2.2H_2O$ is used as a reducing agent $SnCl_2.2H_2O$ will reduce $^{99m}$Tc-pertecnetate ($^{99m}TcO_4$) to $^{99m}$Tc-reduced ($^{99m}TcO_2$) with the following redox reaction:

Tc (VII) + Sn (II) → Tc (V) + Sn (IV)

Tc (V) + Sn (II) → Tc (III) + Sn (IV)

$SnCl_2$ is a reducing agent that is widely used in labeling compounds with radioisotopes. In the process of labeling apigenin with technetium-99m, a sufficient amount of SnCl2 is needed to reduce all $^{99m}TcO_4^-$ to $^{99m}$Tc with a lower oxidation number which will bind to the apigenin ligand. However, the amount of $^{99m}TcO_2$ formed must not be too much because the remaining $^{99m}TcO_4^-$ and $^{99m}TcO_2$ excess can reduce the purity percentage of compounds marked $^{99m}$Tc-Apigenin.[12]

Determination of the $SnCl_2.2H_2O$ conditions of the solution is done using 5 variations in the number of reductor ie 10, 20, 30, 40, and 50 µL, with the formula shown in table 1.

**Table 1: Variation of formulas in $SnCl_2.2H_2O$ optimization.**

| Formula | Apigenin (µL) | pH | $SnCl_2$ (µL) | HCl 0,1 M (µL) | NaOH 0,1 M (µL) | $H_2O$ (µL) | $TcO_4^-$ (µL) | Incubation Time (min) | End Vol (mL) |
|---|---|---|---|---|---|---|---|---|---|
| **A** | 600 | 6 | 10 | - | 20 | 120 | 250 | 30 | 1 |
| **B** | 600 | 6 | 20 | - | 30 | 100 | 250 | 30 | 1 |
| **C** | 600 | 6 | 30 | - | 40 | 80 | 250 | 30 | 1 |
| **D** | 600 | 6 | 40 | - | 50 | 60 | 250 | 30 | 1 |
| **E** | 600 | 6 | 50 | - | 60 | 40 | 250 | 30 | 1 |

The results of the five formulas in the reducting agent ($SnCl_2.2H_2O$) variations can be seen the average radiochemical purity results shown in Table 2 and Figure 2.

**Table 2: Percentage of radiochemical purity of variations in $SnCl_2.2H_2O$.**

| $SnCl_2$ (mg) | 0.1 µL | 0.2 µL | 0.3 µL | 0.4 µL | 0.5 µL |
|---|---|---|---|---|---|
| Radiochemical Purity (%) | 64.39±1.86 | 74.88±1.52 | 74.27±0.01 | 72.68±0.32 | 66.83±3.40 |
| $TcO2^-$ (%) | 16.61±9.10 | 21.99±2.02 | 19.75±1.56 | 25.67±1.77 | 21.95±9.24 |
| $TcO4^-$ (%) | 19.00±10.96 | 3.13±0.49 | 5.99±1.56 | 1.65±2.00 | 11.22±8.63 |

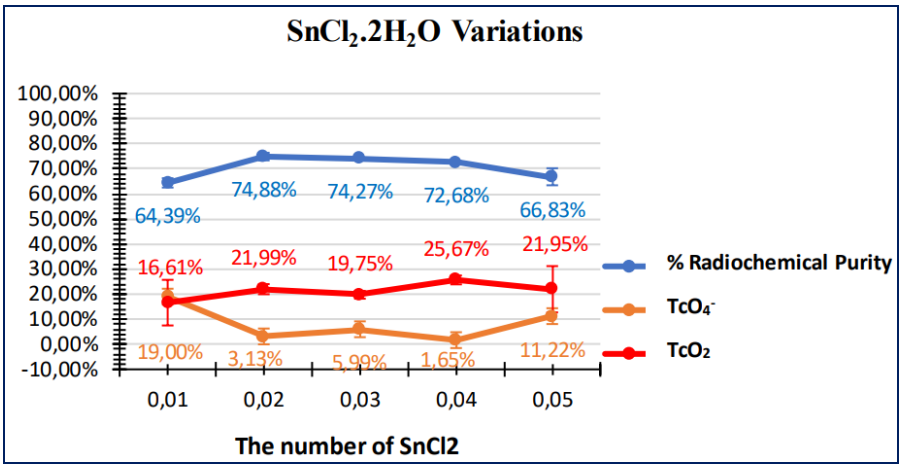


**Fig. 2: Radiochemical purity in $SnCl_2.2H_2O$ variations.**

The results of the optimization of the $SnCl_2.2H_2O$ reducing agent obtained the highest percentage of purity with the amount of $SnCl_2.2H_2O$ of 0.02 mg, and the radiochemical purity value of 74.88% ± 1.86%. The purity percentage is obtained from the reduction of $TcO_4^-$ and $TcO_2$ impurities.

In the amount of 0.01 mg $SnCl_2$ reducing agent, the value of the percentage of $TcO_4^-$ is still quite high when compared to other amount variations, which is equal to 19.00% ± 10.96%.

This is because the amount of $SnCl_2$ added to the sample is too small, as a result there is still a lot of $TcO_4^-$ that has not been reduced. Whereas in the amount of 0.05 mg $SnCl_2$ the percentage of $TcO4^-$ again increased and the percentage of $TcO_2$ decreased. This might be due to the addition of a greater volume of NaOH to obtain pH 6 or optimum pH, making it possible to form precipitate [Sn (OH) 3]$^-$. The presence of these deposits will reduce the reactivity of $Sn^{2+}$ so that the reduced amount of $TcO_4^-$ is reduced.[10]

## CONCLUSIONS

The results of the labeling of apigenin compounds with Technesium-99m radioactive obtained the optimum in the number of reducting agent ($SnCl_2.2H_2O$) conditions is 0.02 mg with radiochemical purity of 74.88% ± 1.86%.

## REFERENCES

1. M. F. Mahomoodally, A. Gurib-Fakim, and A. H. Subratty, "Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius," Pharmaceutical Biology, 2005; 43(3): 237–242.
2. 72. A. Mishra, A. K. Sharma, S. Kumar, A. K. Saxena, and A. K. Pandey, "Bauhinia variegata leaf extracts exhibit considerable antibacterial, antioxidant and anticancer activities," BioMed Research International, vol. 2013, Article ID 915436, 10 pages, 2013.
3. C. T. Ho, T. Osawa, M. T. Huang, and R. T. Rosen, Food Phytochemicals for Cancer Prevention II. Teas, Spices, and Herbs, American Chemical Society, Oxford University Press, 1994.
4. A. K. Pandey, "Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed Parihenium histerophorus: an in vitro study," National Academy Science Letters, 2007; 30: 11-12, 383–386.
5. E. H. Kelly, R. T. Anthony, and J. B. Dennis, "Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships," Journal of Nutritional Biochemistry, 2002; 13(10): 572–584.
6. A. K. Pandey, A. K. Mishra, and A. Mishra, "Antifungal and antioxidative potential of oil and extracts derived from leaves of Indian spice plant Cinnamomum tamala," Cellular and Molecular Biology, 2012; 58: 142–147.
7. G. Cao, E. Sofic, and R. L. Prior, "Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships," Free Radical Biology and Medicine, 1997; 22(5): 749–760.

8. Early, P. J., Sodee, D. B. (1995) Principles and Practice of Nuclear Medicine. Mosby, Toronto.
9. Hladik III, W. B., Saha, G. B., Study, K. T. (1987) Essentials of Nuclear Medicine Sciences. Williams and Wilkins, Sydney.
10. Bernardo-Filho, M., Pires, E. T., Boasquevisque, E. M., Hassón-Voloch, A. (1993) Studies on the incorporation of technetium-99m to the platyhelmint Dugesia tigrina. Rev. Parasitol, 1993; 44: 7–11.
11. Saha, G. B. (2003) Fundamentals of Nuclear Pharmacy. Springer-Verlag, New York.
12. Zolle, I. Technetium-99m Pharmaceuticals: Preparation and Quality Control. New York: Springer Berlin Heidelberg New York, 2007.
13. Owunwanne, A., Patel, M., Sadek, S. The Handbook of Radiopharmaceuticals. London: Chapman & Hall Medical, 2012.
14. Cushnie, T.P.; Lamb, A.J. Antimicrobial Activity of Flavonoids. International Journal of Antimicrobial Agents, 2005; 26: 343–356.
15. Middleton, E.; Kandaswami, C.; Theoharides, T.C. The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease, and Cancer. Pharmacological Reviews, 2000; 52: 673–751.
16. Celen S, de Groot T, Balzarini J et al (2007) Synthesis, evaluation of a (99m)Tc-MAMA-propyl-thymidine complex as a potential probe for in vivo visualization of tumor.