Preparation of Rutin Labeled Scandium-46 and Optimization of Its Planar Chromatography Analysis

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ABSTRACT

Aim: This study aims to perform [^{46}Sc]Sc-Rutin complex as natural product labeled radioisotope compound together with optimization of its planar chromatography analysis.

Methods: Rutin was directly mixed and reacted with [^{46}Sc]$ScCl_3$ after dissolved in methanol or water. A series of variation of molar ratio between rutin and [^{46}Sc]$ScCl_3$ solution was investigated to find optimum condition of [^{46}Sc]Sc-Rutin complex compound. Formed [^{46}Sc]Sc-Rutin complex and unreacted scandium-46 was separated by various chromatography system in order to determine optimum planar chromatography system in [^{46}Sc]Sc-Rutin analysis.

Results: In this study, TLC system with methanol and 10% ammonium acetate (w/w) as mobile phase and 10 cm Whatman-31ET chromatographic paper as stationary phase was found as optimum planar chromatography system for [^{46}Sc]Sc-Rutin analysis. Retardation factor (Rf) of [^{46}Sc]$ScCl_3$ and unreacted scandium-46 were 0 – 0.125 and 0.9 respectively. Variations of molar ratio Sc : rutin at 4:1, 3:1, 2:1, 1:1, 1:2, 1:3 and 1:4 were resulted in optimum molar ratio at 1:2 with 99.16% labeling yield

Conclusion: In summary, rutin could react directly with scandium-46 radioisotope and resulted in [^{46}Sc]Sc-Rutin labeled compound. Further experiment will be carried out to improve labeling yield and to assess other characteristics of [^{46}Sc]Sc-Rutin labeled compound including physico-chemical, pharmacological and biological examination.

Keywords: rutin, radiolabeled compound, scandium, planar chromatography

INTRODUCTION

Rutin (rutoside or vitamin P) and its complexes have been investigated for its pharmacological effects such as anticancer effect and chemotherapeutic activity. Rutin was found in *Ruta graveolens* plant, and the name of rutin was came from this plant. Main features of rutin structure are cinnamoyl group (Band I) and benzoyl group (Band II) as

shown in Figure 1. Antioxidant and radical-scavenging activities of flavonoid, a group of chemical where rutin belong, play a significant role of rutin as an anticancer agent (1). A significant decrease of HL-60 cancer cell's size in which 120 mg/kg of rutin was administered in a murine model, showed a potential antileukemic activity of rutin (2). However, some results explained rutin was effective mostly as supplement in cancer therapy for instance, rutin has the effect in restoring chemosensitivity of human breast cancer cell to overcome multi drug resistance problem in cancer treatment (3), or the ability of rutin to increase thyroid uptake by decrement of serum T4 and T3 and increment of sodium iodide importer activity (4).

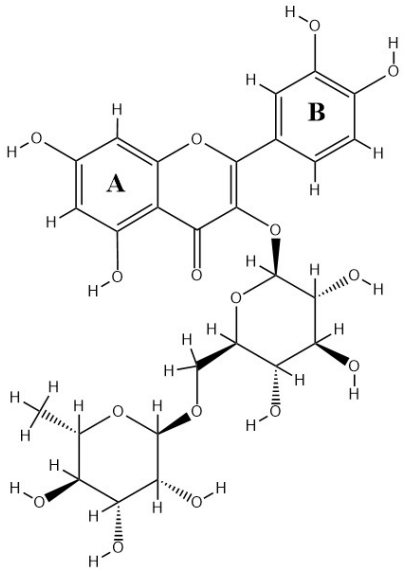

Figure 1. Structure of Rutin with Ring A as benzoyl group (Band II) and Ring B as cinnamoyl group (Band I)

Direct involvement of rutin and flavonoid in anticancer activity has been shown in several researches of rutin-metal complexes, but very few in sole rutin. Rutin-zinc(II) complex indicated cytotoxicity in leukemia cell nevertheless non complexed rutin has no activity against leukemia (5), while Rutin-vanadium and rutin-chrom(III) complexes shown potential antioxidant activity higher than free rutin (6)(7). Another study, with quercetin as a study object instead of rutin, indicated cytotoxicity effect in several cancer cells when quercetin-lanthanide complexes were administered to the cancer cell (8). There are several other studies about anticancer activity of rutin and rutin complex, however it is still difficult to understand the mechanism of rutin or rutin complex when anticancer effect happened.

Thus, radiolabeled compound, a compound that was reacted with radioactive, could help in understanding biodistribution and pharmacokinetics of rutin or rutin complex, especially in animal model. For instance, radiolabeled rutin has been studied to determine biodistribution of rutin in animal model, rutin was successfully labeled with technetium-99m and main uptake of [^{99m}Tc]Tc-Rutin was found in kidney (9), but if rutin was labeled with iodine-125, different route of biodistribution would be found, most of rutin was accumulated at liver and small intestine (10). [^{99m}Tc]Tc-Rutin radiolabeled compound is defined as rutin complex whereas ^{125}I-rutin still has exact structure with normal rutin compound, only a group in the rutin structure substituted with iodine-125 radioactive atom. [^{99m}Tc]Tc-Rutin could be used in bio-study of rutin complex, but short half-life of technetium-99m, only 6 hours, could be a matter for prolonged study, such as pharmacokinetic study and long-term therapy monitoring.

Radioisotope with longer half-life could be used in radiolabeled rutin complex to overcome short half-life problem in pharmacokinetic study or long-term therapy monitoring.

Scandium-46, gamma (889 keV, 1121 keV) and beta emitter (111.8 keV) radioisotope with 83 days of half-life, could be a suitable radioisotope for radiolabeling and biodistribution study of rutin complex to overcome short half-life of ^{99m}Tc. Several studies in natural product radiolabeling with scandium-46 has been carried out for instance [^{46}Sc]Sc-kumarin (11) and [^{46}Sc]Sc-bleomycin (12). Altough the method of [^{99m}Tc]Tc-rutin and [^{46}Sc]Sc-radiolabeled compounds has been investigated, the experiment of [^{46}Sc]Sc-rutin is still yet attempted elsewhere.

This work will describe the preparation of [^{46}Sc]Sc-rutin radiolabeled compound, along with optimization of its planar chromatography for monitoring the labeling yield of rutin with ^{46}Sc. Optimized planar chromatography method is required in Sc:rutin molar ratio determination as an important part of [^{46}Sc]Sc-rutin physico-chemical characteristics. Also the optimized method would be developed for further quality control protocol of [^{46}Sc]Sc-rutin radiolabeled compound.

MATERIALS AND METHODS

Materials and Apparatus

Scandium oxide (Sc_2O_3) with 99.999% purity was purchased from Aldrich. Hydrochloric acid (HCl) 37% p.a, sodium hydroxide (NaOH) p.a, ammonia solution (NH_3) p.a, DTPA, ammonium acetate were obtained from Merck. Aluminium irradiation tube (in house fabricated) with 2.5 cm diameter and 10.5 cm height was used as target container for irradiation. This irradiation container was equipped with aluminium cap matched with the system in TRIGA 2000 research reactor Bandung.

Hitatchi 12-120 double beam UV-Visible Spectrophotometer with tungsten and deuterium lamp, wavelength range of 190 nm – 370 nm (UV) 370 nm – 820 nm (visible) was used to determine UV-Vis characteristics of Sc-rutin. Resolution of wavelength selector is 0.1 nm.

Dose calibrator (Biodex Atomlab 500 plus) was utilized in radioactivity dose determination. Single channel analyzer with NaI(Tl) detector (Ortec) was used for chromatographic paper counting process. Each chromatographic papers to be counted were cut per 1 cm and inserted into detector chamber. Time for each counting was 4 seconds. Window energy for counting process was set in ^{46}Sc energy peak (889 keV).

Multi channer analyzer with high purity germanium (HPGe) detector (Canberra) was used for radionuclidic purity determination of [^{46}Sc]$ScCl_3$ radioisotope solution. Measurement and analysis was conducted using Canberra software. Efficiency and energy accuracy of HPGe detector was annually calibrated by standard point source.

[^{46}Sc]$ScCl_3$ Irradiation and Preparation

100 mg Sc_2O_3 was weighed and inserted into aluminium irradiation tube and subsequently placed in CT-5 irradiation position of TRIGA 2000 Research Reactor. Neutron flux in that position was 2.16 x 10^{12} $n.s^{-1}.cm^{-2}$. Irradiation process was carried out for 3 days. After 24 hours of cooling time, irradiation tube was transported into processing box. Irradiated Sc_2O_3 target was taken out from irradiation tube and transferred into 100 mL beaker glass prior to addition of 40 mL 3N HCl and stirred for 5 hours with gentle heating (60^{o}C – 70^{o}C). Stirring

and heating process were carried out until final volume of [^{46}Sc]$ScCl_3$ was reduced to 30 mL. Radionuclidic purity of [^{46}Sc]$ScCl_3$ was determined by gamma spectrometry.

Rutin Stock Solution Preparation

A total of 3 mg of rutin was placed in 10 mL glass vial and 6 mL of ethanol was subsequently added. Vial was capped and shaken by vortex mixer for 5 min until clear solution of rutin was formed. Similar procedur was applied for rutin solution in water solvent instead of solubilty of rutin in water is less than in ethanol. 1 mg of rutin should be added with 10 mL water in order to obtain aqueous solution of rutin.

[^{46}Sc]Sc-Rutin Preparation and Molar Ratio Optimization

Several solutions with different mole ratio was prepared in the 10 mL glass vial. Selection of mole ratio regarded other study about metal(III)-rutin or metal(III)-quercetin complexes, for instance Cr(III)-rutin complex (6) with provisional molar ratio Cr : rutin 2:1 and rare metal-quercetin complex (8) with proposed molar ratio 1:3. Based of both results, we estimated that stoichiometric molar ratio of Sc : rutin was between 2:1 and 1:3. Molar ratio variety and composition of each ratio was showed in Table 1.

Table 1: Molar Ratio and Composition of [^{46}Sc]$ScCl_3$ (Sc) and Rutin (R)

Molar Ratio Sc:R	Volume added (µL)			
	[^{46}Sc]$ScCl_3$	Rutin Stock	H_2O	Total Volume
4:1	66	1000	3434	4500
3:1	49.5	1000	3450.5	4500
2:1	33	1000	3467	4500
1:1	16.5	1000	3483.5	4500
1:2	16.5	2000	2483.5	4500
1:3	16.5	3000	1483.5	4500
1:4	16.5	4000	483.5	4500

Planar Chromatography Optimization

Three different mobile phases and three different stationary phases were subjected in planar chromatography analysis to find optimized system of [^{46}Sc]Sc-Rutin analysis. This optimized system is important to assess molar ratio optimization of [^{46}Sc]Sc-Rutin. Optimized system should have separate unreacted [^{46}Sc]ScCl3 and rutin from formed [^{46}Sc]Sc-Rutin in thin layer chromatographic system. The compositon of chromatography systems used in this study was showed in Table 2.

Table 2: Planar Chromatography Optimization

Step	Mobile Phase	Stationary Phase
1st	10 mM DTPA	Whatman-31ET
1st	4% Ammonia in H_2O	Whatman-31ET
1st	10% Ammonium acetate in metanol	Whatman-31ET
2nd	Selected A, B or C	Whatman-31ET
2nd	Selected A, B or C	TLC-SG F60
2nd	Selected A, B or C	ITLC-SG

Specificity of Peaks in Planar Radiochromatogram

After radiochromatogram has been obtained, retardation factor (Rf) dan resolution factor (Rs) between two peaks were taken into consideration in TLC optimization determination. Retardation factor (Rf) was determined as ratio of analyte migration distance versus solvent migration distance (13), while resolution factor (Rs) was determined as

> *Rs* = 2 × (distance between the centers of two adjacent spots) / (sum of the two spots in the direction of development) (14).

Higher Rf means analyte favors to mobile phase than stationary phase, whereas higher Rs means two adjacent peaks are analytically separated.

UV-Vis Spectra Determination of Sc-Rutin Complex

To assess UV-Vis characteristics of Sc-Rutin complex, rutin, $ScCl_3$ and Sc-Rutin solution were measured in Hitachi double UV-Vis Spectrophotometer using identical quartz glass cuvette. Absorbance was plotted against wavelength. The composition of sample solution for UV-Vis determination was explained in Table 3.

Table 3: Sample Preparation for UV-Vis Characteristics Determination

Sample	Volume added (µL)			
	$[^{46}Sc]ScCl_3$	Rutin Stock	H_2O	Total Volume
2:1	33	1000	3467	4500
1:2	16.5	2000	2483.5	4500
1:4	16.5	4000	483.5	4500
$ScCl_3$	16.5	0	4483.5	4500
Rutin	0	2000	2500	4500

RESULTS AND DISCUSSION

$[^{46}Sc]ScCl_3$ Radioisotope Solution

Dissolution process of post irradiated Sc_2O_3 was taken 3 hours of 60^{o}C heating and stirring. Final solution of ^{46}Sc radioisotope was $[^{46}Sc]ScCl_3$ with radionuclidic purity up to 99.9% by gamma spectrometry analysis and radiochemical purity up to 99.2% by paper chromatographic method described by other study (12). Total radioactivity obtained in final solution was 21.5 mCi measured by dose calibrator and radioisotope concentration of $[^{46}Sc]ScCl_3$ was 0.72 mCi / mL.

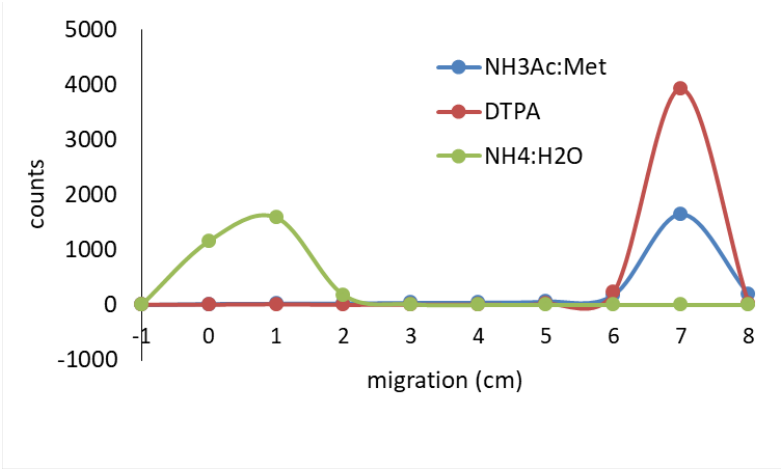

Figure 2. Radiochromatogram of [^{46}Sc]$ScCl_3$ in whatmann-31ET stationary phase with (red) 10mM DTPA (green) 4% ammonia in water and (blue) 10% ammonium acetate : methanol

TLC chromatogram of [^{46}Sc]$ScCl_3$ to assess radiochemical purity of radioisotope solution was represented in Figure 2. For green graph, stationary phase was whatman-31ET and 4% ammonia in water (w/w) as mobile phase. Rf of [^{46}Sc]$ScCl_3$ peak in this study was in accordance with other study despite different stationary phase was used (15). Red graph depicts [^{46}Sc]$ScCl_3$ peak at Rf 0.9 when mobile phase of 10mM DTPA was used combined with whatmann-31ET paper. According to report from Moghaddam (12), ^{46}Sc formed a more lipophilic [^{46}Sc]Sc-DTPA complex and carried by the mobile phase to the solvent-front positon. If mobile phase of the chromatographic system was changed to 10% ammonium acetate:methanol mixture, [^{46}Sc]$ScCl_3$ peak shifted to solvent-front position at Rf 0.9 (blue graph). From the three chromatogram systems analysis, it was found that radiochemical purity of [^{46}Sc]$ScCl_3$ was higher than 95% for each system

Planar Chromatography Optimization

Several chromatographic systems was evaluated in this study to find a suitable system to identify the formation of [^{46}Sc]Sc-Rutin complex. The chromatographic system should be able to separate [^{46}Sc]Sc-Rutin from unreacted ^{46}Sc. Three kind of mobile phases were examined as first step to find ideal mobile phase for scandium species separation. Optimization of planar chromatography analysis continued to the second step. Selected mobile phase in the first step would be used to examine three kind of stationary phase as the second step of planar chromatography analysis optimization.

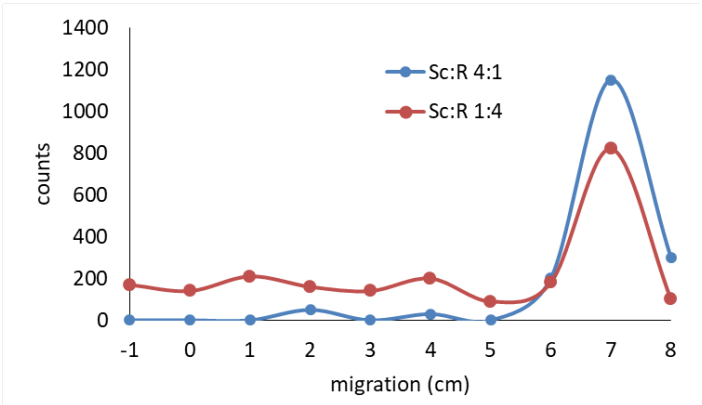

Figure 3. Radiochromatogram of [^{46}Sc]$ScCl_3$ in whatmann-31ET stationary phase and 10mM DTPA mobile phase (red) molar ratio Sc:R 1:4 and (blue) molar ratio Sc:R 4:1

Figure 3 shows radiochromatogram of [^{46}Sc]Sc-Rutin in 10 mM DTPA mobile phase. Molar ratio of ^{46}Sc : rutin was 1:4 (red) and 4:1 (blue) for excess of rutin and excess of ^{46}Sc respectively. Both of molar ratio have similar pattern of peak at Rf 0.9. Those peaks position were close to free ^{46}Sc peak as depicted in picture 4.A. Raised background, monitored in -1 to 6 cm position in Sc:R 1:4, might be signal of [^{46}Sc]Sc-Rutin formation. In conclusion, 10 mM DTPA mobile phase could not separate [^{46}Sc]Sc-Rutin and unreacted ^{46}Sc. Single peak appeared at the solvent front position of radiochromatogram.

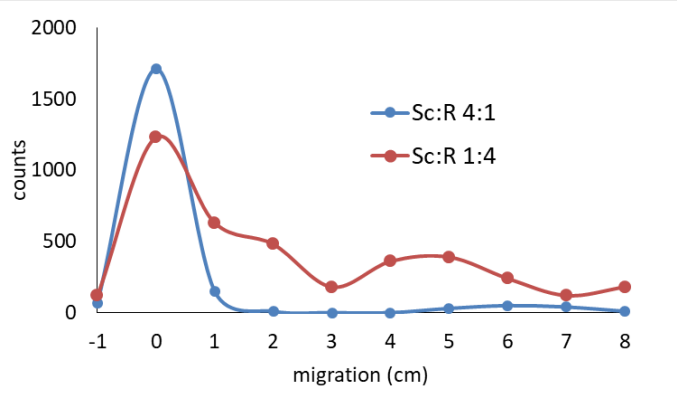

Figure 4. Radichromatogram of [^{46}Sc]$ScCl_3$ in whatmann-31ET stationary phase and 4% ammonia:water mobile phase (red) molar ratio Sc:R 1:4 and (blue) molar ratio Sc:R 4:1

Two obvious peaks were appeared in radiochromatogram with 4% ammonia in water mobile phase as shown in red graph of Figure 4 First peak was observed as fronting peak with Rf at 0 represents free ^{46}Sc, while broad second peak has the Rf at 0.625 represents [^{46}Sc]Sc-Rutin. However, in blue graph while excess ^{46}Sc existed in solution, second peak of [^{46}Sc]Sc-Rutin became smaller and almost leveled with background.

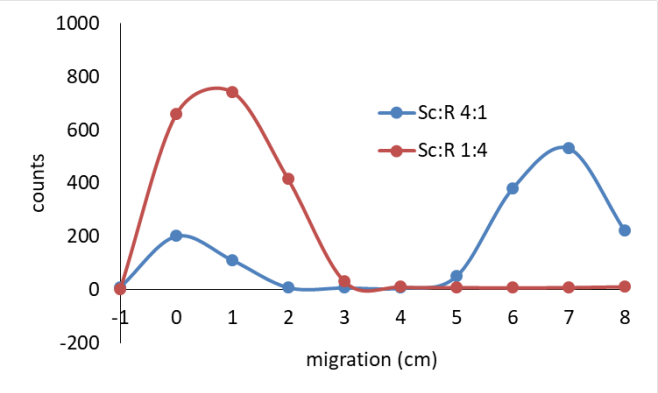

Figure 5. Radiochromatogram of [^{46}Sc]$ScCl_3$ in whatmann-31ET stationary phase and 10% ammonium acetate:methanol mobile phase (blue) molar ratio Sc:R 1:4 and (red) molar ratio Sc:R 4:1

Ideal separation of labeled rutin as [^{46}Sc]Sc-Rutin and unreacted scandium-46 was depicted in Figure 5. If excess rutin was mixed with ^{46}Sc, broad peak was observed at origin position of radiochromatogram (red), there was no peak at Rf 0.9 in red graph as free unreacted ^{46}Sc peak because all of ^{46}Sc reacted with rutin and resulted in [^{46}Sc]Sc-Rutin formation. While in Figure 5, second peak was observed at solvent front position in Rf 0.9. There was larger portion of unreacted ^{46}Sc with molar ratio ^{46}Sc:rutin 4:1 and the second peak represented

unreacted free ^{46}Sc. Rf of second peak in Figure 5 has similar Rf of ^{46}Sc stock solution in 10% ammonium acetate : methanol mobile phase (Figure 4). Unlike in 4% ammonia:water, [^{46}Sc]Sc-Rutin peak still appeared clearly in 10% ammonium acetate:methanol inspite of excess ^{46}Sc in the [^{46}Sc]Sc-Rutin solution. [^{46}Sc]Sc-Rutin complex remained in initial point of radiochromatogram because it was stable enough and did not undergo any ligand exchange with acetate. Free ^{46}Sc formed a complex with acetate and move apart from intial point together with ammonium acetate mobile phase.

1st step of TLC analysis optimization was summarized in Table 4 below. It can be concluded that 10% ammonium acetate:methanol was the best mobile phase to separate Sc-rutin and unreacted free scandium regarding of resolution factor (Rs) between Sc-rutin and free scandium peaks.

Table 4. Summary of Rf and Rs in Mobile Phase Optimization

Mobile Phase	Rf / position (cm)		Peak width (cm)		Rs
	[^{46}Sc]Sc-Rutin	^{46}Sc	[^{46}Sc]Sc-Rutin	^{46}Sc	
10 mM DTPA	0.9 / 7	0.9 / 7	2	2	0
4% Ammonia : H_2O	0.625 / 5	0.1 / 0	4	4	0.625
10% Ammonium acetate : metanol	0 / 0	0.9 / 7	3	3	1.17

According to resolution factor result of first step in Table 4, 10% ammonium acetate:methanol was regarded as selected mobile phase because of its ability to separate Sc-rutin peak and unreacted free scandium peak. Then, this mobile phase was used in second step of stationary phase optimization. Three stationary phases were examined, those were whatman-31ET, TLC-SG and ITLC-SG. Table 5 shows the Rf and Rs of each stationary phase. TLC-SG and ITLC-SG generated similar pattern of radiochromatogram. Unreacted free ^{46}Sc slightly moved from initial spot point whereas [^{46}Sc]Sc-Rutin was bound in initial point with silica in TLC/ITLC-SG as seen in Figure 6. ^{46}Sc has higher mobility in whatman-31ET paper far from initial spot point compared to mobility in TLC/ITLC-SG. ^{46}Sc was retarded in silica because it has more polarity compared to moiety of water content in cellulose of whatman-31ET while free rutin moved together with ammonium acetate : methanol solvent to the end point of radiochromatogram as seen in Figure 6. Rutin as flavonoid is less polar than ^{46}Sc. Based on the result in Table 4 and Table 5, whatman-31ET and 10% ammonium acetate:methanol solvent were considered as optimized planar chromatographic system to analyze radiochemical purity of [^{46}Sc]Sc-Rutin labeled compound.

Table 5. Rf and Rs in Stationary Phase Optimization

Stationary Phase	Rf / position (cm)		Peak width (cm)		Rs
	[^{46}Sc]Sc-Rutin	^{46}Sc	[^{46}Sc]Sc-Rutin	^{46}Sc	
TLC-SG	0 / 0	0.25 / 2	2	4	0.33
ITLC-SG	0 / 0	0.125 / 1	2	4	0.33
Whatman-31ET	0 / 0	0.9 / 7	3	3	1.17

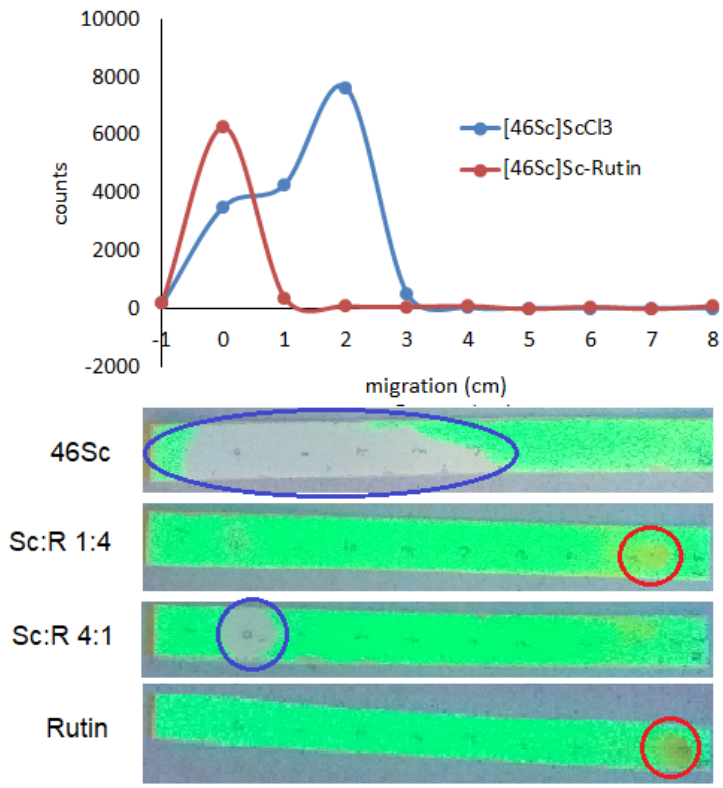

Figure 6. Radiochromatogram of [^{46}Sc]Sc-Rutin and [^{46}Sc]$ScCl_3$ in TLC-SG with 10% ammonium acetate : methanol solvent (top) Post elution of TLC-SG paper as seen under UV light

[^{46}Sc]Sc-Rutin Molar Ratio Optimization

Several studies about flavonoid complexes with some metals have been carried out. In complexation of rare earth metal (RE(III)) with quercetin, it was proposed that the complex would be 1:3 in rare earth-quercetin molar ratio (8). Other study about cerium (III) and rutin reaction suggests that one molecule of rutin acted as electron donor with two cerium molecules (6). In this study, we found that one scandium molecule bound with two rutin molecules according to Figure 7. ^{46}Sc was utilized as radiotracer to monitor reaction process between scandium and rutin. Amount of [^{46}Sc]Sc-Rutin formed and residual ^{46}Sc were analyzed by planar chromatography described before. In 1:2 molar ratio of Sc:R, residual amount ^{46}Sc was less than 1% because almost all scandium reacted with rutin as seen in Figure 7. Addition of excess scandium more than 1:2 molar ratio would decrease percentage of [^{46}Sc]Sc-Rutin and increase residual ^{46}Sc, this condition could be seen from molar ratio 1:1 to 4:1, whilst addition of more rutin did not has any significant effect of [^{46}Sc]Sc-Rutin percentage, the labeling yield of [^{46}Sc]Sc-Rutin remained high (more than 99%).

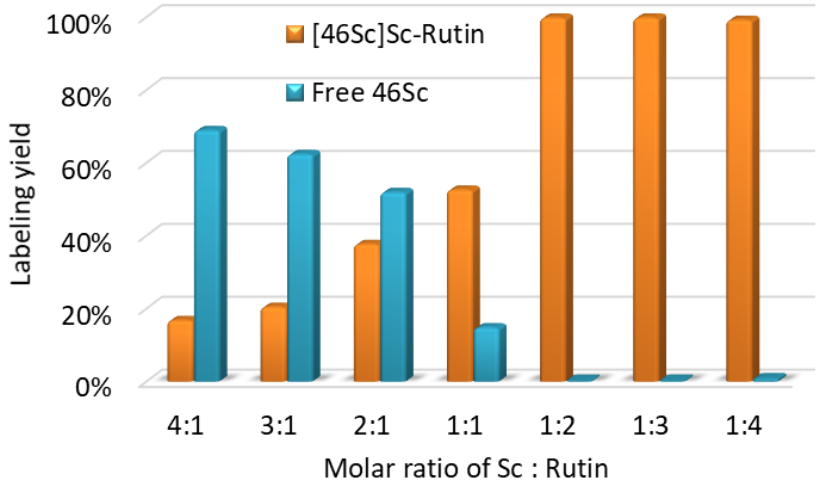

Figure 7. Effect of molar ratio to labeling yield of [^{46}Sc]Sc-Rutin

According to the optimized molar ratio of Sc:rutin, there are three possibilities of scandium-rutin complex in molar ratio 1:2. First and second, metal-oxygen bond was formed between two benzoyl group of rutin and two cinnamoyl group of rutin respectively. Third possibility was mixed position of metal-oxygen bond between benzoyl and cinnamoyl bond (Figure 8).

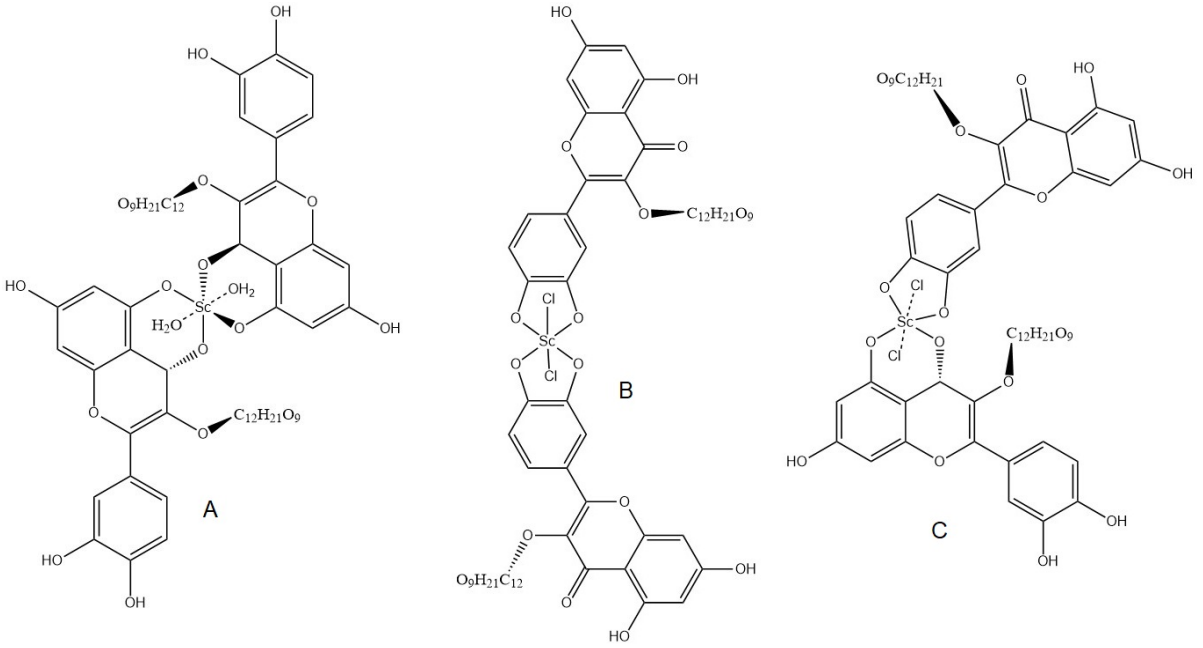

Figure 8. Three possible structure of Sc-Rutin Complex with coordination bond of scandium and oxygen between (A) benzoyl group, (B) cinnamoyl group and (C) mixed both groups.

CONCLUSION

[^{46}Sc]Sc-rutin radiolabeled compound has been successfully prepared by direct labeling of rutin in room temperature by shaking for 30 min. Optimum molar ratio of scandium : rutin in Sc-rutin complex was investigated in 1:2 molar ratio. Whatman-31ET chromatographic paper combined with 10% ammonium acetate:methanol as solvent were ideal planar

chromatography system for speciation of free scandium as ^{46}Sc and formed [^{46}Sc]Sc-rutin radiolabeled compound. Further experiment will be carried out to improve labeling yield and to assess other characteristics of [^{46}Sc]Sc-Rutin labeled compound including physico-chemical, pharmacological and biological examination.

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