Synthesis of ^{131}I Labeled Quercetin through Oxidation Method Using Chloramine-T for Cancer Radiopharmaceuticals

Maula Eka Sriyani[1,*], Dian Ayu Utami[2], Mega Dwike Susilo[2], Eva Maria Widyasari[1], Muharram Marzuki[2], Rizky Juwita Sugiharti[1], and Witri Nuraeni[1]

[1]*National Nuclear Energy Agency, Jl. Tamansari No. 71 Bandung 40132, Indonesia*

[2]*Jenderal Achmad Yani University, Jl. Terusan Jenderal Sudirman Cimahi, Indonesia*

* ***Corresponding author:***

email: maula@batan.go.id

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Abstract: *Quercetin is one of the flavonoid groups with antioxidant activity. The objective of this study was to achieve the labeled compound of ^{131}I-quercetin as a radiotracer for diagnosis and cancer therapy with high labeling efficiency and radiochemical purity. The labeling procedure was conducted by the oxidation reaction using chloramine-T. The effect of pH, reaction time, amount of oxidizing agent and ligand were evaluated in this research. Quercetin was successfully labeled with iodine-131 at pH 11 at room temperature for 10 min mixed in 1000 rpm with the amount of quercetin and chloramine-T is 0.4 and 0.3 mg, respectively. The results demonstrated that the ratio of quercetin/Na^{131}I was 2 × 10^5. The ^{131}I-quercetin labeling efficiency was 92.03 ± 2.20%, and radiochemical purity of ^{131}I-quercetin was 99.34 ± 0.58%. The results showed that ^{131}I-quercetin could be a radiotracer candidate for diagnosis and cancer therapy.*

Keywords: *^{131}I-quercetin; iodination; natural compound; synthesis; anticancer*

■ INTRODUCTION

Cancer is one of the cellular diseases where abnormal cells grow irrepressibly [1]. According to the data from Indonesian Ministry of Health, cancer caused about 8.2 million of death in 2012, and the number of new cases is expected to rise by about 70% over the next 2 decades [2-3]. Nowadays, 30–50% of cancer incidents can be prevented by avoiding risk factors and implementing existing evidence-based prevention strategies. Some of the cancers patients will survive if it was diagnosed early and treated adequately. Therefore, the burden of cancer suffering can also be reduced [4].

Nuclear techniques using radiopharmaceuticals offer the alternative method to detect cancer in the early stage of the disease. The contribution of nuclear techniques to cancer therapy is in terms of early disease detection, staging, therapy selection, and follow-up to personalized medicine [5]. In the part of therapy, the selection of nuclear technique or nuclear medicine offers therapy known as radio-metabolic. The radio metabolic consists of radionuclides that emit a "curative" type of radiations such as beta- or alpha-particles [6]. One of the most commonly used radionuclides for targeted radiotherapy is iodine-131(^{131}I). ^{131}I is easy in availability, low cost with the physical half-life for 8.04 days, with beta rays emission (max 606 keV) and linear energy transfer in tissue of 2.5–3 mm. It was expected to be distributed in blood and tissue to destroy the cancer cells. ^{131}I also emits gamma (364 keV) rays that suitable to detect from outside the body using gamma camera [7].

Flavonoids are mostly present in nature in the form of benzo-γ-pyrone derivatives. they can be found in a variety of plants, vegetables, and flowers. Flavonoids have diverse structural frameworks and play important roles in the body's defense system [8]. Quercetin is one of the flavonoid compounds that have antioxidant, antiviral, anticancer, anti-inflammatory and hepatoprotective activity [9-11]. Based on Free-Wilson techniques on quantitative structure analysis relationship, quercetin has 88.77% of antioxidant activity [11]. The results showed that quercetin has a high potential as an anticancer. The antioxidants characteristics of quercetin

could inhibit the carcinogenesis process due to cancer [12]. These antioxidants activity characteristics mainly due to the double bond of C2 and C3 and also hydroxyl groups in C3 that could inhibit the oxidation process. The antiradical activity also could be affected by the hydroxyl group at C4'. The antiradical activity is also increased by the *ortho*-hydroxyl system at B ring [13].

Iodine-131 is very useful for radiolabeling of flavonoid structures because it can be easily introduced to a phenolic group on the substrate with a small structural alteration from the original substrate [14]. The research on quercetin labeling using iodine-131 was initially conducted by Barolli and Pomilio [15]. The radioiodination of quercetin was conducted in two different methods, the thallation, and oxidation using chloramine-T. The first method gave 2'-iodinated product while the second method gave poly-substitution product of 2',6,8-triiodo-quercetin [15]. In 2011, another research was conducted on the labeling of quercetin using ^{125}I, and it has shown that the apoptosis capability was increased to the DU 145 prostate cancer cells to 13% (16).

^{131}I-quercetin is a radiolabeled natural compound that is expected to be a radiotracer for diagnosing cancer. It has the potential to strengthen and to maximize the curative effect of quercetin itself against cancer. The differences of this research from the previous method were (i) the radionuclide used in this research was Iodine-131 that emits both β and γ radiation and enables it to detect and also cure the cancer cells simultaneously compare to another study by Hosseinmehr et al. [16] and Park et al. [17], (ii) the structure found in this research was different from the other research by Barolli and Pomilio [15], (iii) the research was conducted in an alkaline solution and did not used an organic solvent such as methanol [18]. On the first step, the prediction of the I-quercetin structure using the non-radioactive iodine-127 shown in Fig. 1 was studied [19]. The other research had been conducted to identify the structure of iodinated quercetin [19], the next step was optimizing the labeling condition of quercetin using radionuclide of ^{131}I that was conducted in this research.

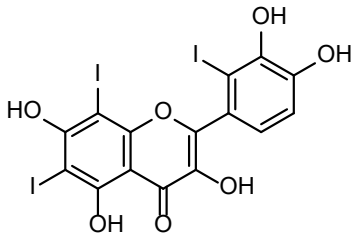

Fig 1. Structure compound of iodoquercetin

■ EXPERIMENTAL SECTION

The purpose of this research was to obtain the optimum condition for the labeling of ^{131}I-quercetin as a radiotracer for diagnosis and cancer therapy with high labeling efficiency and radiochemical purity. ^{131}I was chosen because it emits both γ and β rays. The presence of both emitters enabled the ^{131}I-quercetin to work as a diagnostic agent as well as therapy. The labeling process was conducted through the oxidation of iodine using the chloramine-T method [15]. Several aspects of the formulation of labeling compound were also considered such as the effect of pH of the reaction, oxidizing agent, amount of the ligand and the reaction time.

Materials

Quercetin hydrate, chloramine-T hydrate (Sigma Aldrich), sodium iodide (Na^{131}I) (BATAN), sodium metabisulphite, sodium hydroxide, chloroform, methanol and HCl (E. Merck). NaCl 0.9% (IKA Pharma), TLC-SG F_{254} (E. Merck) and distilled water (IKA Pharma).

Instrumentation

Single Channel Analyzer (Ortec, Model 4890). Dose Calibrator (Victoreen), Magnetic Stirrer (Nouva), analytical balance (Metler Toledo), Shaker (Biotech), Oven (Memmert), Vortex Mixer (Retcsh), Indicator pH Universal (E. Merck), Syringe (Terumo), separating funnel, micropipette, tip, vial 10 mL, microtube 2 mL, lead container, and other glassware.

Procedure

In a series of microcentrifuge tubes (1.5 mL), different amounts of quercetin were dissolved in NaOH 0.03N (2 mg/mL). Then, fresh chloramine-T in distilled

water (75–600 µg) was added to the quercetin solution. The pH was then adjusted from 8.0–12.0 followed by addition of 50 µL I-131 (~37.5 MBq). The reaction was ended by the addition of sodium metabisulphite (100–500 µg). The total volume of the reaction was 1000 µL. In a separating funnel, 1 mL of chloroform was added to the mixture to wash away free iodine via extraction. This procedure was repeated for three times. The radioactivity counts of the chloroform and aqueous phase were respectively measured by dose calibrator in order to calculate the radiolabeling efficiency.

The radiochemical purity of ^{131}I-quercetin was determined using TLC-SG F_{254} plastic sheet. As much as 5 µL of reaction mixture from the aqueous phase was placed on the start line, then chromatographed using methanol p.a. as a developing system. The strips were removed, dried and cut into 1 cm segments and assayed for radioactivity using Single Channel Analyzer.

Radiochemical purity and labeling efficiency are determined as requirement characteristics for radiopharmaceutical. The radiochemical purity was determined by thin layer chromatography (TLC) silica gel F_{254} as a stationary phase and methanol as mobile phase. I^- as a radiochemical impurity was at Rf 1.0. The radiochemical purity was determined using Eq. 1, 2 [20]:

$$\%I^- (\text{Impurity}) = \frac{\text{counts on Rf 1}}{\text{total counts}} \times 100\ \% \quad (1)$$

$$\%\,^{131}\text{I-Quercetin} = 100\% - \%I^- (\text{Impurities}) \quad (2)$$

The labeling efficiency was calculated using the Eq. 3:

$$\%\ \text{Radiolabeling efficiency} = \frac{\%\ \text{radioactivity after labeling}}{\%\ \text{radioactivity before labeling}} \quad (3)$$

■ RESULTS AND DISCUSSION

In the labeling of quercetin, there are some factors of labeling that have to be considered including the amount of ligand (quercetin), the oxidizing agent (chloramine-T) and incubation time. Moreover, the labeling condition is affected by pH. The pH of the reaction should be optimized to achieve high radiochemical purity. Radiochemical purity requirements of the ^{131}I labeled compound for cancer treatments is more than 90% [21].

Radioiodination can be done through several methods, including thallation and oxidation with chloramine-T methods. However, the oxidation method using chloramine-T was chosen because this method has been widely used by several other studies with satisfactory results [14,16,18,21]. This method was also chosen because the reaction may be carried out in an alkaline solution (NaOH).

In this research, the radiochemical purity determination was conducted through the TLC chromatographic method. Since the retention factor (Rf) obtained for ^{131}I-quercetin was 0.8, and it was different with the impurities I^-(Rf 1) and I_2 (Rf 0), it did not need any further analysis like previous research [15-16].

The acidity level (pH) is one of the important factors affecting the success of radio-iodination labeling ^{131}I-quercetin. The pH was varied from 8 to 12. In the previous research conducted by Barolli and Pomilio [15] and Xie et al. [18], the reaction has occurred at pH 10 and pH 6, respectively. In this research, the optimum pH was obtained at pH 11 with radiolabeling efficiency of 86.15 ± 1.33% (Table 1) and radiochemical purity of 87.32 ± 6.86% (Fig. 2). The radiolabeling efficiency showed the reaction yield obtained in this study, while the radiochemical purity declared the purity of the compounds obtained.

The iodination reaction had occurred when the chloramine-T oxidized I^- ($Na^{131}I$) and formed I^+ that could bind the ligand. In the basic solution, I^+ would be formed as hypoiodous acid (HOI) and decreased the pH of reaction. Iodination of quercetin by hypoiodous acid was done through electrophilic substitution reaction where I^+ substituted H^+ at the quercetin [23] as mentioned in Eq. 4.

Table 1. Labeling efficiency of iodoquercetin at different pH

pH	Labeling efficiency (%)
8	57.51 ± 8.99
9	76.89 ± 1.69
10	80.32 ± 13.44
11	86.15 ± 1.33
12	82.35 ± 7.09

$$\underset{\text{Quercetin}}{C_{15}H_9O_7} - HOI \rightleftharpoons \underset{Na_2S_2O_5}{C_{15}H_9O_7} - I + HI + H_2O \quad (4)$$

Quercetin has several sites for iodination depending on the reaction condition. For instance, under methyl ether as a blocking group, it will produce 6-iodoquercetin [17]. Whereas at the neutral condition, it will produce 2'-iodoquercetin [15] and 8-iodoquercetin [18]. In this research, the iodination of quercetin produced 5',6,8-triiodo-quercetin as described in Table 2 and Fig. 3.

The incubation was done at room temperature, based on the literature [15]. The time of incubation was varied 10, 20, 30, 40, 50 and 60 min to find out the optimum condition of labeling at the different time interval. The results showed that the good labeling condition was achieved

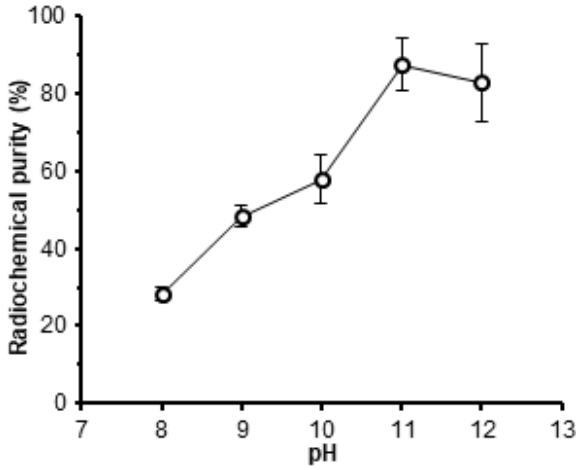

Fig 2. The radiochemical purity of Iodoquercetin at different pH

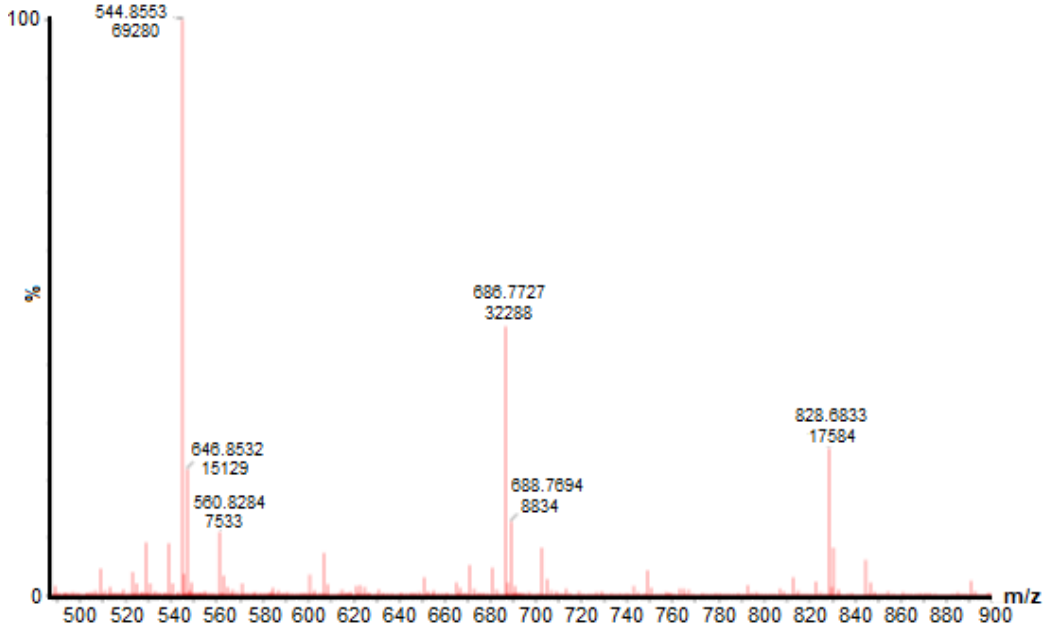

Fig 3. The MS spectra of iodoquercetin

Table 2. Characteristics of quercetin hydrate and iodinated quercetin [19]

No	Physical Characteristics		Quercetin Hydrate	I-Quercetin
1	Color		Yellow	Brown
2	Observed melting point		317 °C	> 600 °C
3	Crystal microscopic form		Bar	Spherulite
4	^{1}H-NMR Chemical shift	H6	6.2 (d, J=3 Hz)	-
		H8	6.4 (d, J=3 Hz)	-
		H2'	7.7 (d, J=3 Hz)	7.78 (d, J=8 Hz)
		H5'	6.8 (d, J= 17 Hz)	-
		H6'	7.6 (dd, J=3 Hz; 3 Hz)	7.43 (d, J=8 Hz)
5	FTIR Spectra		Finger print area (-)	582, 521 and 467 cm^{-1}
6	MS Spectra (m/z)		301.0348	686.7727

at the time interval at 30–60 min with the maximum labeling efficiency of 84.32 ± 8.44% (Table 3) and radiochemical purity of 88.35 ± 9.06% (Fig. 4).

$$\text{rate} = \frac{P}{t} \quad (5)$$

The product of a reaction depends on the reaction rate. While the reaction rate is influenced by time (Eq. 5). The longer the reaction time, the more products are obtained. A decrease in the number of products at the time of 60 min caused by the number of products that have been optimal. Since the reaction is reversible (Eq. 4), the possibility of the reaction shifts back to the left and makes the amount of products decrease.

Another factor affecting the good result of labeling ^{131}I-quercetin is the amount of quercetin. It was varied from 0.2–1.2 mg. The results showed that 0.4 mg of quercetin gave the labeling efficiency of 76.57 ± 4.26% (Table 4.) and the radiochemical purity of 82.01 ± 9.04% (Fig. 5). Based on the results, it can be seen that there was no difference in the radiochemical purity obtained after the addition of 0.4 mg quercetin. Due to the low chemical concentration of Na^{131}I [18], the quercetin needed to be labeled only in a small amount and have reached its optimal value. It means that almost all of the iodine molecules have reacted with the quercetin molecules.

The oxidizing agent (chloramine-T) is the important factor in the iodination of quercetin. An increasing amount of chloramine-T would increase the number of impurities (I_2 and I^-), otherwise, if the amount of oxidizing agent is less, the iodine will not be oxidized. This research also performed the effect of the chloramine-T to the radio-iodination of quercetin. The amount was varied from 0.15 to 0.45 mg. The results

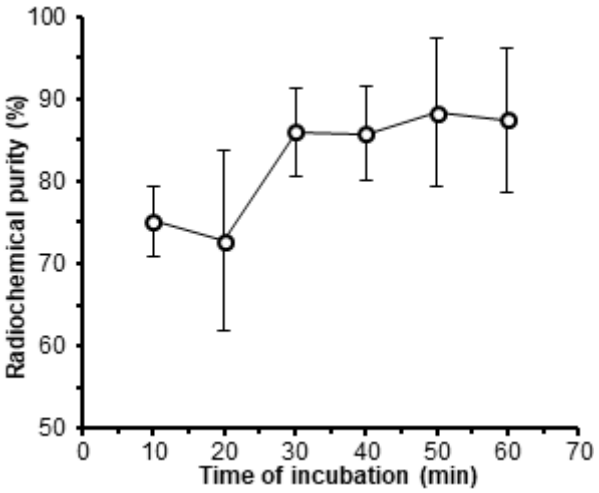

Fig 4. Percentage of radiochemical purity on the variation of incubation time

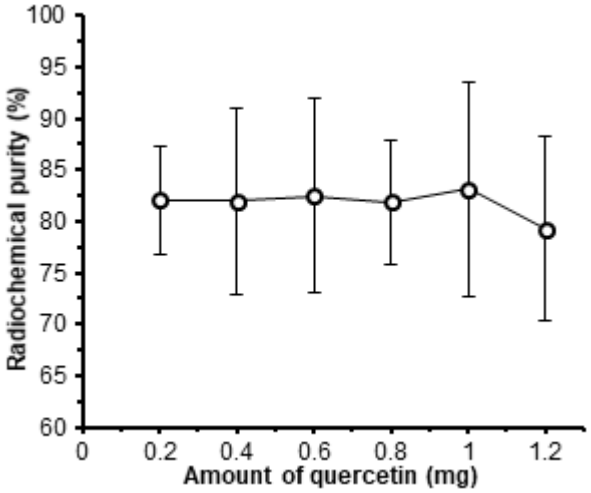

Fig 5. Radiochemical purity of ^{131}I-quercetin at the different amount of quercetin

Table 3. Labeling efficiency on the variation of incubation time

Time of incubation (min)	Labeling Efficiency (%)
10	74.48 ± 4.45
20	84.41 ± 10.02
30	80.85 ± 8.37
40	83.20 ± 4.96
50	84.32 ± 8.44
60	78.99 ± 15.65

Table 4. Labeling efficiency at the different amount of quercetin

Amount of quercetin (mg)	Labeling efficiency of ^{131}I-quercetin (%)
0.2	68.77 ± 9.13
0.4	76.57 ± 4.26
0.6	79.78 ± 8.14
0.8	78.13 ± 5.11
1.0	79.24 ± 5.44
1.2	73.40 ± 9.52

Table 5. Labeling efficiency of ^{131}I-quercetin at the different amount of oxidizing agent

Amount of chloramine T (mg)	Labeling efficiency of ^{131}I-quercetin (%)
0.150	65.36 ± 8.81
0.225	77.65 ± 5.78
0.300	77.64 ± 5.61
0.375	84.50 ± 2.74
0.450	77.17 ± 0.35

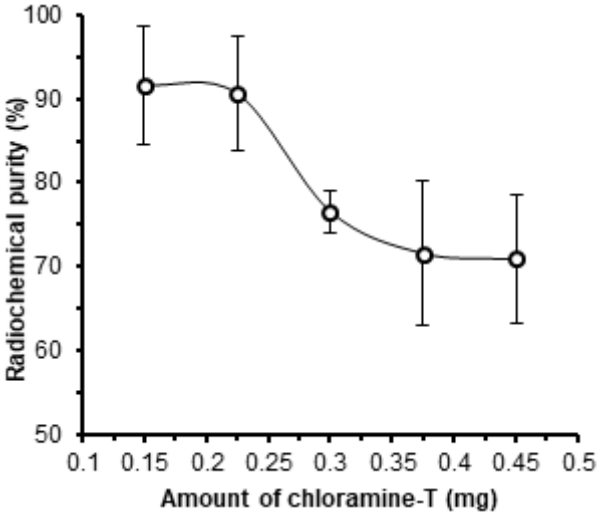

Fig 6. Radiochemical purity of ^{131}I-quercetin on the variation of oxidizing agent

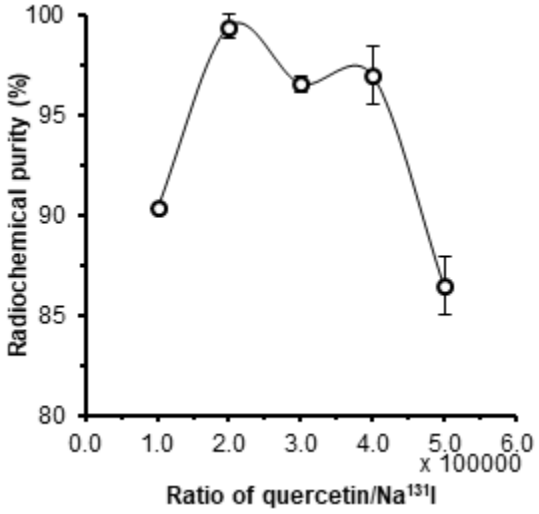

Fig 7. The radiochemical purity of ^{131}I-quercetin on the variation of ratio quercetin/Na^{131}I

showed that the optimum labeling was achieved at 0.225 mg of chloramine-T with the labeling efficiency of 77.65 ± 5.78% (Table 5) and radiochemical purity of 90.01 ± 1.03% (Fig. 6). Chloramine-T plays an important role in the labeling process according to Eq. 4 that the reaction is carried out by the mechanism of electrophilic substitution [22]. Chloramine-T is as an oxidizing agent would oxidize I^- to I^+. I^+ species will bind to the quercetin group which has high electronegativity to form iodine-quercetin compounds.

The next step is the mole ratio between iodine and quercetin. According to Setiawan et al. [23], the moles ratio between the radioisotopes and the ligand is very significantly influenced radiochemical purity of its labeling compound. To conduct this step, firstly we have to quantify the mass of Na^{131}I and quercetin using Eq.6 and 7.

$$Bq = \frac{\text{mass (g)}}{Mr} \times 6.02\times10^{23} \times \frac{Ln\ 2}{t_{\frac{1}{2}}\ (sec)} \quad (6)$$

$$\text{Mole Na}^{131}\text{I} = \frac{\text{mass Na}^{131}\text{I (g)}}{154} \quad (7)$$

The determination of the optimum ratio of quercetin/Na^{131}I was done by examining the value obtained. The ratios were varied from 1 x 10^5 to 5 x 10^5. The highest value shown in Fig. 7 was obtained in the ratio of quercetin/Na^{131}I as 2 × 10^5 with the efficiency of labeling for 92.03 ± 2.20% and radiochemical purity 99.40 ± 0.58%. These results were fulfilled with the requirement of a good ^{131}I labeling compound for cancer (> 95%) [24].

■ CONCLUSION

Quercetin was successfully labeled with Iodine-131 through oxidation method using chloramine-T. The labeling efficiency was 92.03 ± 2.20%, and radiochemical purity of ^{131}I-quercetin was 99.34 ± 0.58%. The optimum results were achieved at pH 11, incubation at room temperature for 10 min mixed in 1000 rpm, and with the amount of quercetin and chloramine-T were 0.4 and 0.3 mg, respectively. The results occurred in the ratio of quercetin/Na^{131}I as much as 2 × 10^5 and met the radiochemical purity requirements for cancer radiopharmaceuticals (> 90%).

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