Heliyon 7 (2021) e08438

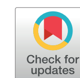
Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Synthesis, stability, and cellular uptake of ^{131}I-estradiol against MCF7 and T-47D human cell lines as a radioligand for binding assay



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ARTICLE INFO

Keywords:
Estradiol
Iodine-131
Estrogen receptor
Chloramine T
Cellular uptake

ABSTRACT

Estradiol is a steroid hormone that works as an agonist estrogen receptor (ER). This compound is widely used as a ligand and bind specifically to the ERα. Radioligand binding assay is an in vitro method for drug development from natural products by synthesizing estradiol through radiolabeling using the radioiodination method. Synthesis of ^{131}I-estradiol was perfomed by direct method using chloramine T as an oxidizer and by indirect labeling using ^{131}I-histamine. The purity of chemical was determined by thin-layer chromatography and paper electrophoresis, as well as its stability for 30 days of storage in refrigerator, freezer and room temperature. The cellular uptake test of the radioligands from both methods was carried out with MCF7 and T-47D cell lines at 60 min. The results exhibited that ^{131}I-estradiol was succesfully obtained with radiochemical purity greater than 95% and more stable in the refrigerator until 21 days than freezer and room temperature. ^{131}I-estradiol and ^{131}I-his-estradiol were internalized higher in T-47D cells than MCF7 cells (44.34 ± 5.93% vs. 17.27 ± 1.71% and 45.34 ± 6.42% vs. 4.92 ± 1.59%, respectively). Furthermore, the radioligands can be used to binding assay in determining the agonist or antagonist to ER of new drugs development.

1. Introduction

Breast cancer is a disease most experienced by women throughout the world [1]. According to data from the World Health Organization (WHO) in 2020, breast cancer worldwide has developed and reached 2.26 million new cases and ranks first of incidence (24.5%) and mortality (15.5%) in women [2]. The number of cases of cancer suffered by women can be predicted from the peculiarities of cancer [3]. Distinctive traits are insulted by unstable genome conditions, implementation of swelling, and genetic uniformity [4, 5]. Factors that trigger a high risk of breast cancer include an unbalanced lifestyle diet, overweight, lack of exercise and smoking habits. Research conducted by Ronco et. al. showed that a low-acid diet directly related to meat intake and inversely proportional to plant-based food intake can reduce the risk of breast cancer [6].

One indicator of breast cancer is Estrogen Receptors (ER) status. There are two types of ER, i.e. positive and negative ER, with differences in the nature of the antiestrogen [7]. Hormone therapy is effective against ER-positive (ER+) tumors, but surgery and chemotherapy are the appropriate therapy against ER-negative (ER−) tumors [8]. Non-invasive examination is currently preferred because of its advantages in breast cancer diagnosis including single-photon emission computed tomography (SPECT), positron emission tomography (PET) scan and magnetic resonance imaging (MRI) [9, 10].

Estradiol is a form of the estrogen hormone that is a steroid hormone of agonist against ER [11]. The effect of estradiol is mediated by ER which is one of the nuclear receptor hormones (intracellular receptors), which will cause proliferation and metastasis [12]. The development of new drugs from synthesis or isolation requires data on molecular binding to receptors. Method that can be used to determine the affinity of drugs

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https://doi.org/10.1016/j.heliyon.2021.e08438
Received 7 August 2021; Received in revised form 20 October 2021; Accepted 16 November 2021
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and the mechanism of binding to receptors at the molecular level is radioligand binding assay (RBA) by functional or radioligand competition binding assays [13]. The advantages of RBA are measure at low receptor concentrations, selective and sensitive [14]. Estradiol is a compound that can be easily to radiolabel and developed using $^{125/131}$Iodine or Tritium (^{3}H) [15]. This radioligand is paired with ER and must be compatible and have the same action or agonist with the receptor [16].

Several studies have been reported on labeling a hormon with various radioisotope. Resveratrol is a flavonoids from natural product which was tested by the RBA method to determine the agonist or antagonist properties of the ER using ^{3}H-estradiol [17]. Katzenellenbogen also investigated fluoroestradiol and fluoro-furanyl-nor-progesterone for breast cancer and have performed binding to receptors using ^{3}H-estradiol [9]. Xu et al. have developed a radioiodinated $^{125/131}$I-estradiol dimer for estrogen receptor-targeted breast cancer imaging [16]. Indirect labeling of β-estradiol-17β-hemisuccinate using histamine with ^{125}I conducted by Susilo et al. resulted in a radiochemical purity of 97.8% [18]. On the other hand, Kumar et. al used ^{18}F-fluoroestradiol to determine the binding to ER through imaging with PET/CT [19]. From all these studies, radiolabeling must meet the requirements of radiochemical purity (RCP) more than 95%, radioligand stability and ability to bind to the desired receptor.

In this study, direct synthesis of estradiol using Iodine-131 will be carried out using the radioiodination method. This synthesis was chosen because it is a relatively simple and easy for radiolabeling, but it is necessary to determine its stability after radiolabeling, due to the relatively short half-life of iodine-131 (8 days). As a comparison, the radiolabeling was also carried out by indirect method following the procedure of Susilo et al [18]. This radioligand was tested for its binding ability with two types of cancer cells to determine the ability of the radioligand to the estrogen receptor in these cells to prove that the radioligand had the same binding ability as the original estradiol.

2. Materials and methods

2.1. Instruments

Microtubes, erlenmeyer, beaker, measuring cup, stirring rod, micropipette (Eppendorf), 0.2 and 0.5 mL microcentrifuge tubes, a set of paper chromatography and paper electrophoresis equipment, analytical balance (Metler Toledo), oven (Memmert), incubator 37 °C (Memmert), digital shaking dry bath (Thermo Scientific), laminar airflow cabinet for radioactive (Comecer), refrigerator -20 °C (Samsung), dose calibrator (Biodex), multi-channel analyzer (Canberra), RadioTLC Scanner (Bioscan), and HPLC UV-Vis and radioactive detector (Agilent Technology).

2.2. Chemicals

β-estradiol (Sigma Aldrich), β-estradiol-17-hemisuccinate (Sigma Aldrich), Na^{131}I (irradiated by Serpong-Siwabessy Multi-Purpose Reactor and processed in the Research Center for Radioisotope and Radiopharmaceutical Technology laboratory, Nuclear Energy Research Organization (BATAN), National Research and Innovation Agency (BRIN), Indonesia), chloramine T (Sigma Aldrich), sodium metabisulfite (Merck), benzene (Merck), ethanol (Merck), chloroform (Merck), disodium hydrogen phosphate (Merck), sodium dihydrogen phosphate (Merck), acetonitrile (Merck), methanol (Merck), demineralized water, universal pH paper, hydrochloric acid (Merck), sodium hydroxide (Merck), and Whatman paper 1 (Sigma Aldrich).

2.3. Cell Culture

MCF7 human breast cancer was obtained from the Central Laboratory, Universitas Padjadjaran, and cell line T-47D was obtained from the Laboratory of Cell Culture and Cytogenetics, Faculty of Medicine, Universitas Padjadjaran.

2.4. Radioiodination of β-estradiol

β-estradiol has been labeled by direct reaction with Na^{131}I using chloramine T (CAT) method. In a 0.2 mL microtube, 1 mg of β-estradiol was dissolved in 0.5 mL of absolute ethanol, then 10–100 μL CAT (1 mg/mL) was added. The microtube was placed into a Pb container, then 5–10 μL of Na^{131}I solution with radioactivity between 1.85-3.7 MBq (50–100 μCi) was added. It was then shaken until homogeneous. The solution was shaken with a vortex stirrer for a period of time from 1-15 min at room temperature. Immediately, 10–100 μL of sodium metabisulfite solution with a concentration of 2.5 mg/mL was added to stop the reaction.

2.5. Selection of chromatographic system in determination of radiochemical purity of ^{131}I-β-estradiol

The radiochemical purity of ^{131}I-estradiol was determined by ascending paper chromatography and electrophoresis methods. Paper chromatography used six variations of the stationary phase, Whatman 1 and Whatman 3 MM with various mobile phases; methanol:water (90:10) and (70:30); TLC SG stationary phase and mobile phase; benzene:ethanol (1:1); benzene:ethanol:acetic acid (75:24:1); chloroform:ethanol (1:1). These two methods were chosen to separate ^{131}I-estradiol from impurities $^{131}I_2$ and free Iodine (131I-). As a comparison, the test was carried out with paper electrophoresis. Cellulose acetate paper was used as stationary phase and Whatman 1 using phosphate buffer solution (0.1 M, pH 7.4) as electrolytes. Electrophoresis was carried out for 60 min at 200 V. The paper for paper chromatography and TLC were dried for 5 min in an 80 °C oven. Then, this paper was counted using RadioTLC Scanner. The data were processed by Excel to calculate the percentage of radiochemical purity of ^{131}I-estradiol in the solution. ^{131}I-estradiol labeled compounds meet the requirements for radiochemical purity if the percentage is ≥95%.

2.6. Optimization of labeling ^{131}I-estradiol

2.6.1. Effect of incubation of reaction time

The optimum incubation time was determined by varying the incubation period at 1, 5, and 15 min at room temperature. The radiochemical purity of ^{131}I-estradiol was determined by paper chromatography using Whatman 1 and methanol:water (90:10) as a mobile phase, which was then counted by TLC-Scanner.

2.6.2. Effect of CAT amount

The initial optimization stage was carried out by varying the concentration of CAT from 10-1000 μg with a fixed incubation time of 5 min. The radiochemical purity of ^{131}I-estradiol was determined by chromatography paper using Whatman 1 and methanol:water (90:10) as a mobile phase, which was then counted by TLC-Scanner.

2.6.3. Effect of estradiol amount

Estradiol was varied from 2, 10, 20, 50, and 100 μg with the optimum amount of CAT and 5 min incubation time. The radiochemical purity of ^{131}I-estradiol was determined by chromatography paper using Whatman 1 and methanol:water (90:10) as a mobile phase, which was then counted by TLC-Scanner.

2.7. Stability test of ^{131}I-estradiol

Stability test of ^{131}I-estradiol was carried out by varying the storage time and temperature. The labeled compound of ^{131}I-estradiol was divided into 10 tubes, each of 5 μL. A total of 10 tubes each were stored at room temperature (25 °C), refrigerator (2 °C) and freezer

(−22 °C). The ^{131}I-estradiol solution was stored at room temperature for a time period of 1–30 days. The test was carried out by the chromatographic method and the percentage of radiochemical purity was calculated.

2.8. Radiochemical purity of ^{131}I-estradiol with RadioHPLC

A total of 10 μL samples of ^{131}I-estradiol solution were injected into the HPLC with a UV and radioactive detector. The eluent used was a mixture of acetonitrile and water (55:45) with varied flow rates of 0.5, 0.75, and 1 mL/min using C-18 column and detection at $\lambda =$ 280 nm. The peak of the labeled compound ^{131}I-estradiol was compared to the estradiol and Iodine-131. The percentage of radiochemical purity and chemical purity was calculated based on the ^{131}I-estradiol peak compared to the impurity peak by UV and radioactive detector.

2.9. Radioiodination of β-estradiol-17-hemisuccinate [18]

Histamine (concentration 2 mg/mL) was dissolved in 0.5 M phosphate buffer pH 8.0 in a micro tube. Then, it was radioiodinated with Na^{131}I (radioactivity 1.85–0.37 MBq (50–100 μCi)). A total of 10 μL of chloramine T (5 mg/mL) that had been dissolved in sterile water was added to the solution and reacted for 2–3 min using a vortex stirrer. The reaction was stopped by adding 10 μL of sodium metabisulfite (300 mg/mL) in sterile water. Activation of estradiol-17β-hemisuccinate was done by dissolving 1 mg of estradiol-17β-hemisuccinate in 50 μL of dioxane followed by adding 20% of tributylamine and 10% isobutyl chloroformate dissolved in dioxane. The solution was stirred using a magnetic stirrer until homogeneous for 30 min at 4–8 °C. Then, the reaction was stopped by adding dioxane. An activated estradiol-17β-hemisuccinate was added to the iodination tube containing ^{131}I–histamine and was incubated for two hours at 4–8 °C. The results of the conjugation of estradiol-17β-hemisuccinate with ^{131}I-histamine were then tested by electrophoresis using Whatman paper no. 1 and 0.025 M phosphate buffer electrolyte pH 7.4 at a voltage of 200 V for 60 min. The purification of ^{131}I-estradiol-17β-hemisuccinate-iodohistamine was carried out by the extraction method using toluene. The organic phase was collected. The radiochemical purity of the purified ^{131}I-estradiol-17β-hemisuccinate-iodohistamine (^{131}I-his-estradiol) was then tested by electrophoresis using Whatman paper no. 1 and 0.025 M phosphate buffer electrolyte pH 7.4 at a voltage of 200 V for 60 min.

2.10. Cellular uptake studies of ^{131}I-estradiol and ^{131}I-his-estradiol

The MCF7 cell line was obtained from the Central Laboratory of Universitas Padjadjaran and prepared beforehand by incubating the cells in RPMI medium for 24 h in a 24 well plate. Cell line T-47D was obtained from the Laboratory of Cell Culture and Cytogenetics, Faculty of Medicine, Universitas Padjadjaran. T-47D cells were prepared in advance by incubating the cells in RPMI medium for 72 h in a 24 well plate. Cells that were confluent in 24 well plates were removed one by one with a micropipette, then rinsed with 1 mL HBSS. The 10μL of sample solution consisting of ^{131}I-estradiol, ^{131}I-his-estradiol, and standard ^{131}I was added into the triple well. The incubation at room temperature was done with the selected time range of 10, 30, and 60 min. One mL of HBSS was added and the HBSS solution was discarded to remove any remaining radioactive compounds. Then, the cells were lysed with the addition of 60μL of 0.2N NaOH. Then, the radioactivity of each cell lysate was measured with an automatic gamma counter. Cell uptake from samples was analyzed by comparing them against standards (% cellular uptake).

This research methodology can be seen in the flowchart in Figure 1.

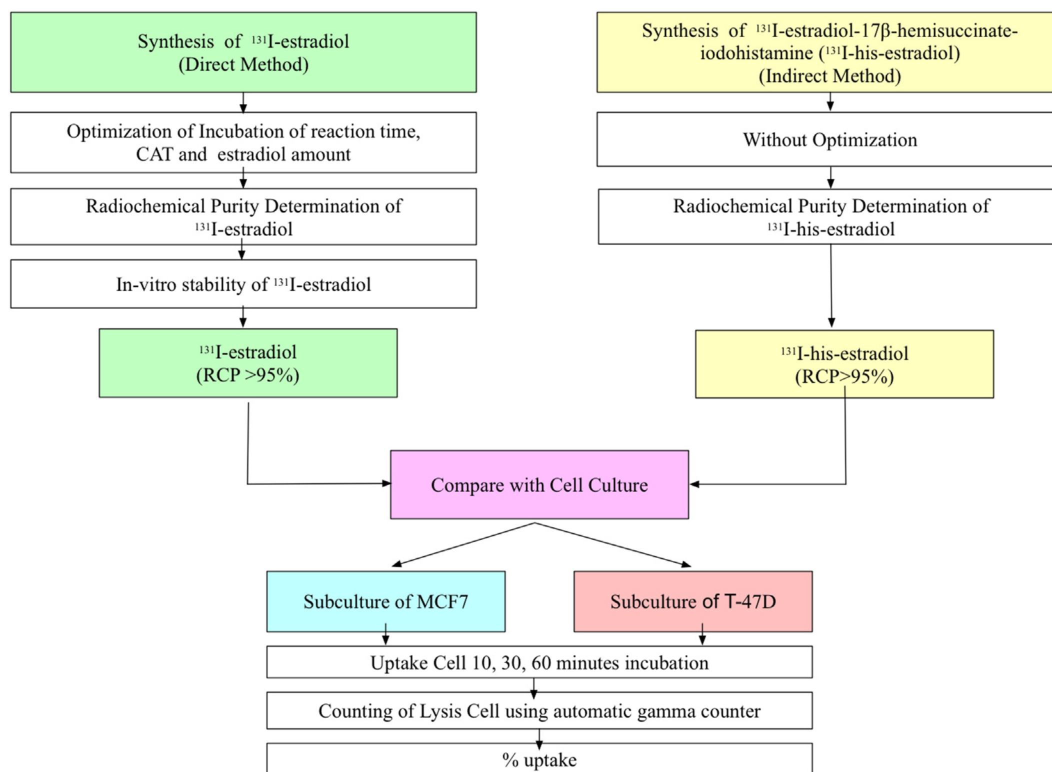


Figure 1. Flowchart of research methodology.

3. Results

3.1. Radioiodination of β-estradiol

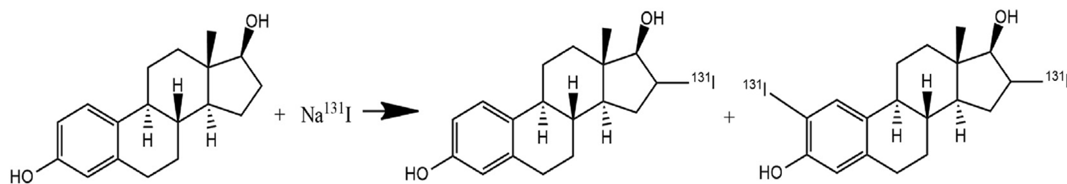


Figure 2. Proposed radioiodination of estradiol reaction.

3.2. Selection of chromatographic system in determination of radiochemical purity of ^{131}I-estradiol

Table 1. Chromatography system selection for determination of radiochemical purity of ^{131}I-estradiol.

Chromatography System		Retention Factor (Rf)	
Stationary phase	Mobile Phase	^{131}I$^-$	^{131}I-estradiol
Whatman 1	Methanol: Water (90:10)	0.8–0.9	0.0–0.2
TLC-SG	Benzene: Ethanol (50:50)	0.8–0.9	0.8–0.9
TLC-SG	Benzene: Ethanol: Acetic Acid (75:24:1)	0.0–0.2	0.8–0.9
Whatman 1	Methanol: Water (70:30)	0.8–0.9	0.0–0.2
TLC-SG	Chloroform: Ethanol (90:10)	0.0–0.2	0.8–0.9
Whatman 3 MM	Methanol: Water (90:10)	0.8–0.9	0.0–0.2

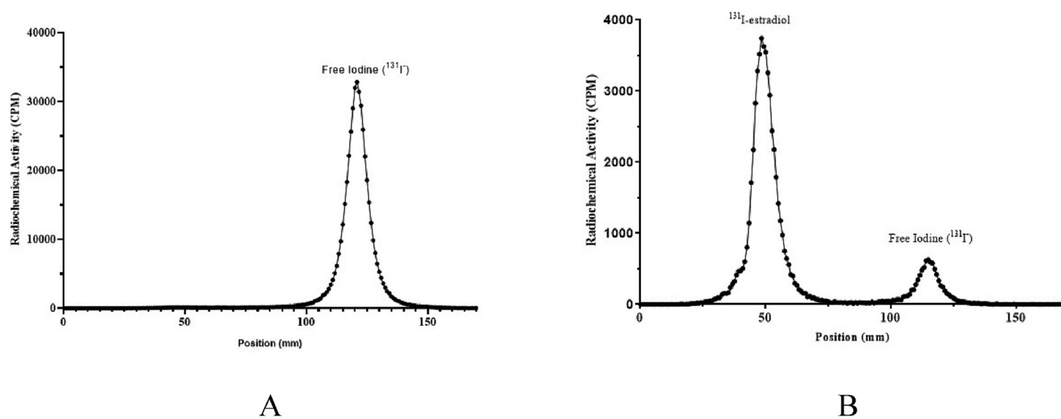


Figure 3. Radiochromatogram profile of Iodine-131 (A) and ^{131}I-estradiol (B) Whatmann 1 as stationary phase and Methanol: Water (90 : 10) as mobile phase.

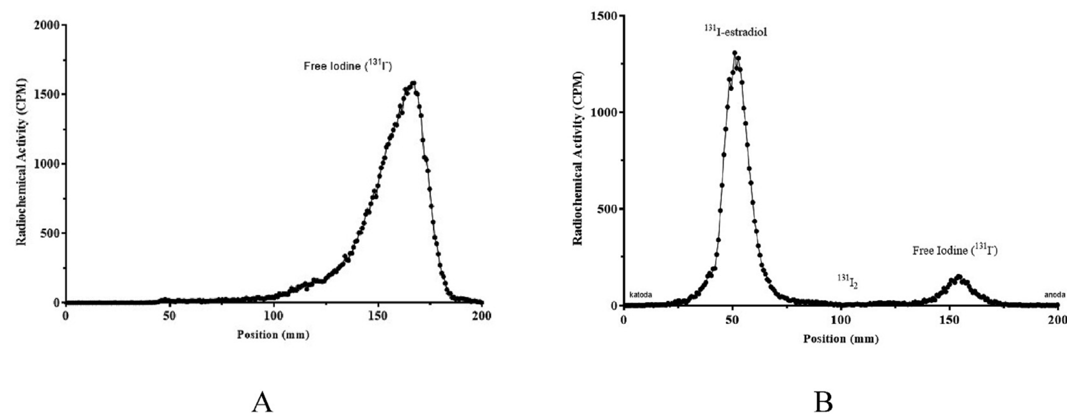


Figure 4. Radiochromatogram profile of Iodine-131 (A) and ^{131}I-estradiol (B) electrophoresis using Whatmann 1 as stationary phase and Phosphate Buffer (0,1 M, pH 7.4) as electrolyte.

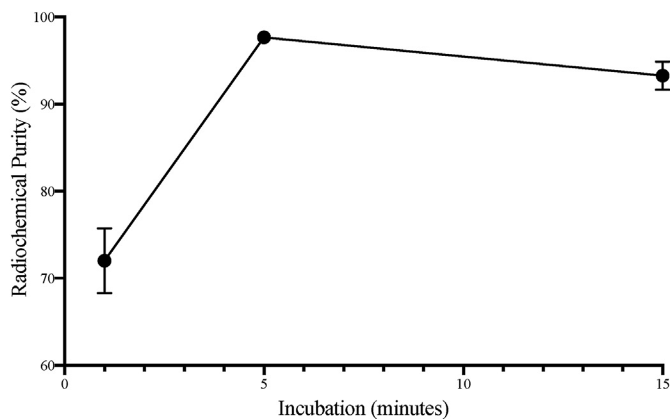


Figure 5. Variation of radiochemical purity of ^{131}I-estradiol of incubation time of the reaction.

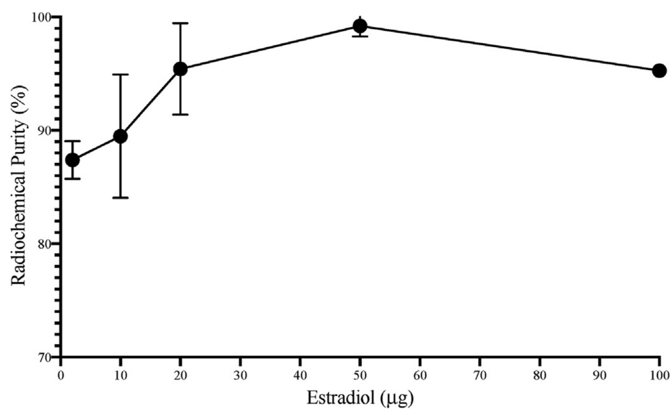


Figure 7. Variation of radiochemical purity of ^{131}I-estradiol of estradiol amounts.

3.3. Optimization of labeling ^{131}I-estradiol

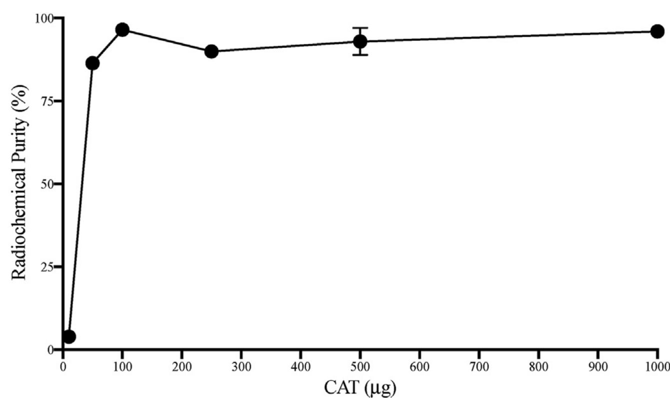


Figure 6. Variation of radiochemical purity of ^{131}I-estradiol of CAT amounts.

3.4. Stability test of ^{131}I-estradiol

The stability test of ^{131}I-estradiol was carried out for 30 days at room temperature (25 °C), refrigerator (2–5 °C) and freezer (−20 °C). The results exhibited that ^{131}I-estradiol was stable in refrigerator (R^2: 0.4454) with radiochemical purity higher than 95 % until 21 days of storage. However, the radiochemical purity higher than 95% was only obtained after 8 days at room temperature and freezer storage as described in Figure 8.

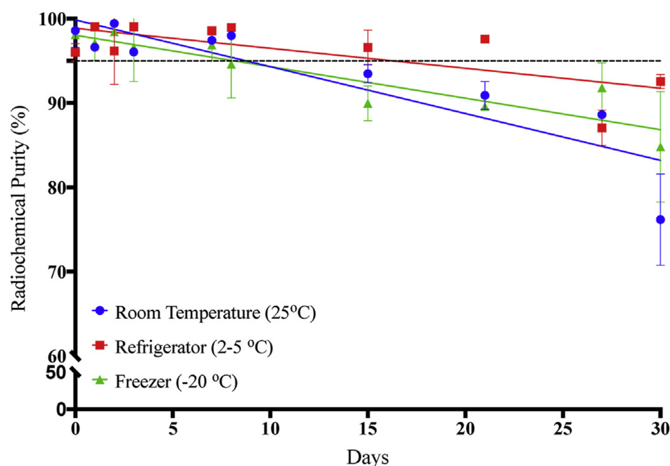
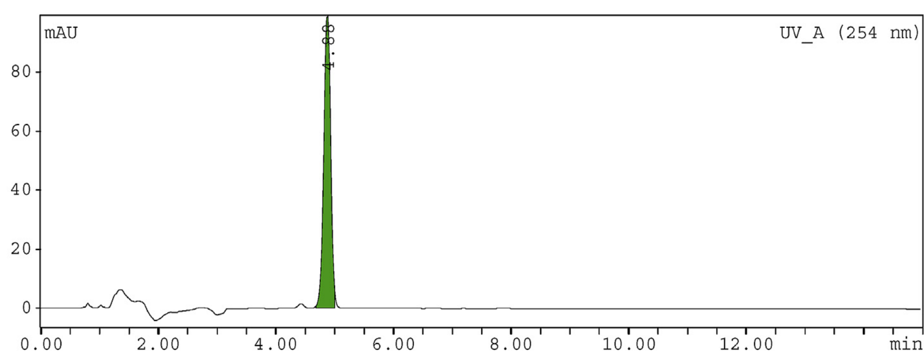
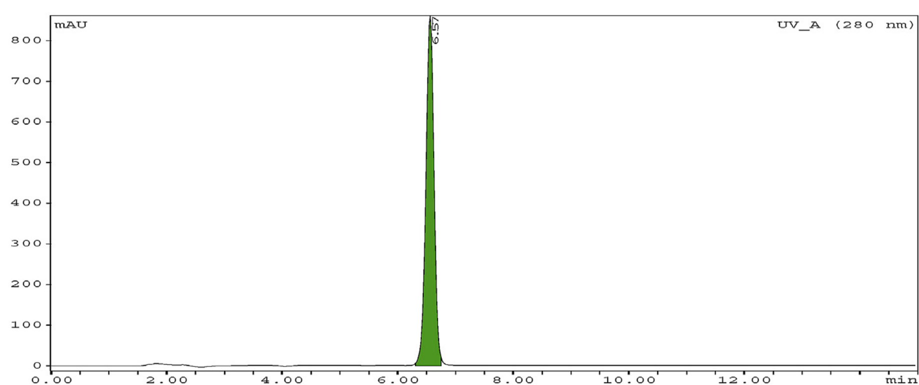


Figure 8. Stability test of ^{131}I-estradiol in several temperatures and storage periods.

3.5. Radiochemical purity of ^{131}I-estradiol with radioHPLC



A



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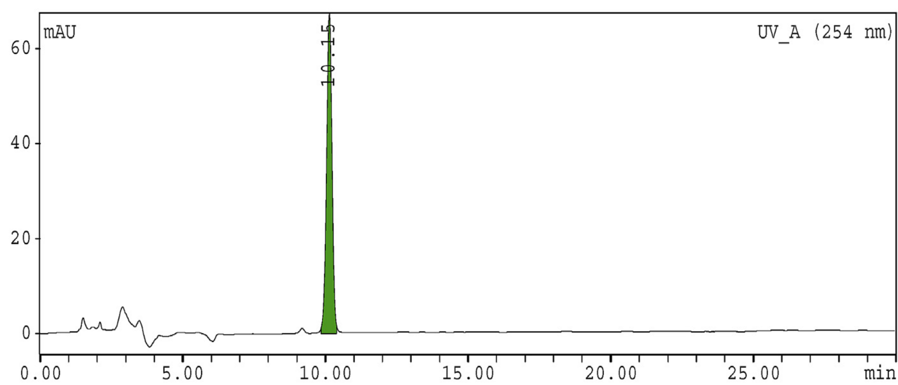

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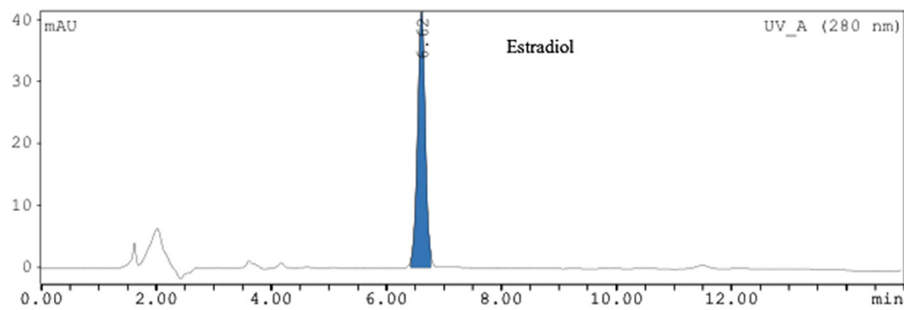
Figure 9. Chromatogram profile of estradiol by radioHPLC (C-18 column, ACN:Water (55:45), flow rate 0.5 (A); 0.75 (B) and 1.0 (C) mL/min).



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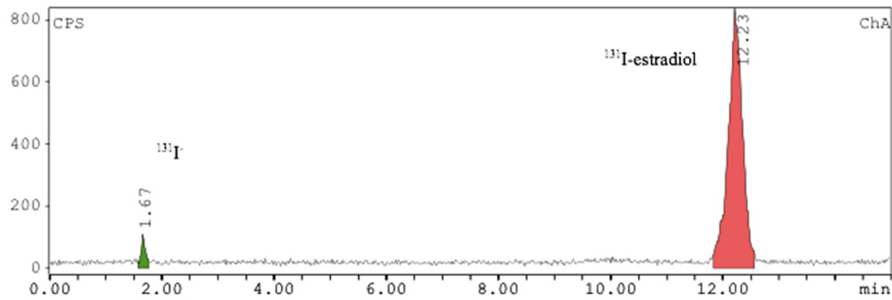

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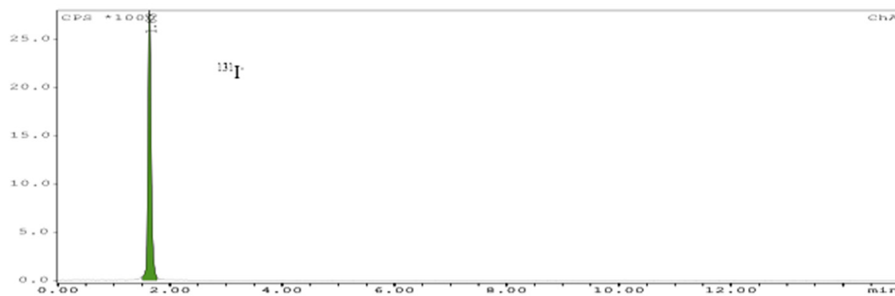

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Figure 10. Chromatogram profile of estradiol (A) ^{131}I-estradiol (B) and $^{131}I^{-}$ (C) by radioHPLC (C-18 column, ACN:Water (55:45), flow rate 0.75 mL/min).

3.6. *Radioiodination of β-estradiol-17-hemisuccinate*

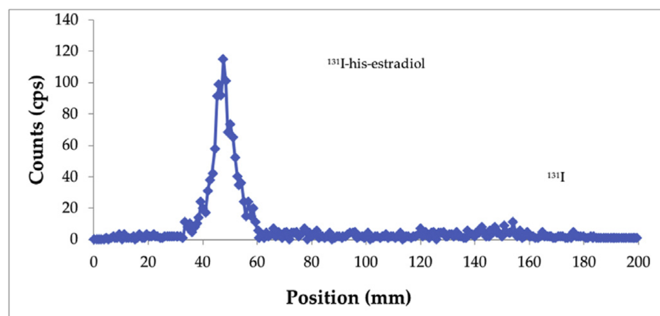

Figure 11. Radiochromatogram profile ^{131}I-his-estradiol (B) electrophoresis using Whatmann 1 as stationary phase and Phosphate Buffer (0.1 M, pH 7.4) as electrolyte.

3.7. *Cellular uptake studies of ^{131}I-estradiol and ^{131}I-his-estradiol*

Cellular uptake test was carried out on estradiol labeled Iodine-131 through direct and indirect labeling with histamine and iodine-131 as control. Our results exhibited both radiolabeled of estradiol was accumulated in MCF7 and T-47D cell lines as described in Figure 12.

Subsequently, the accumulation of both ^{131}I-estradiol and ^{131}I-his-estradiol in MCF7 and T-47D cell lines was stable until 60 min ($p > 0.05$) as illustrated in Figure 13 (a) and each radiolabeled compound was significantly higher in T-47D cell lines compared to MCF7 ($p < 0.01$) (Figure 13 (b)).

4. Discussion

Radioiodination is a method to label substances using γ-emitting radioisotopes of iodine such as ^{125}I and ^{131}I. This method able to produced labeled compounds with high specific radioactivity at very low

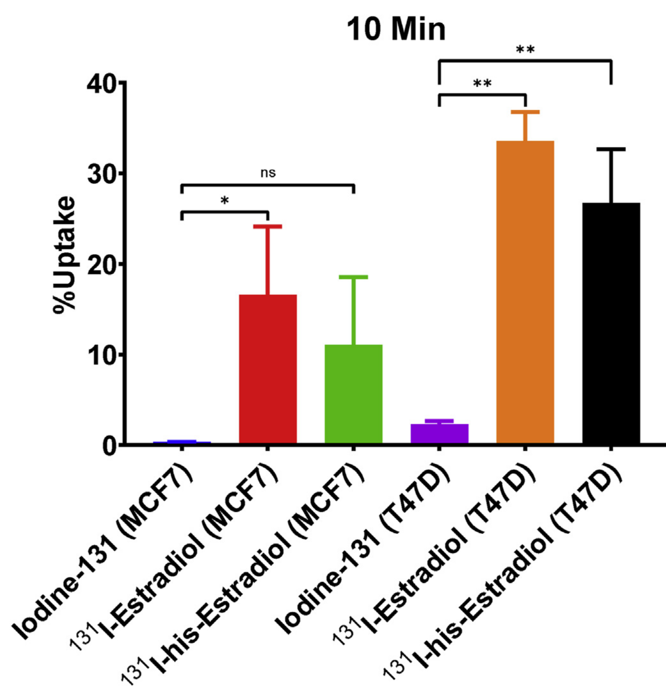

Figure 12. Cellular uptake comparison of ^{131}I-Estradiol and ^{131}I-his-Estradiol to Iodine-131 as negative control.

concentrations [20]. Sodium iodide (Na^{131}I) as I^- was oxidized to I^+ by chloramine T, then it formed complexes with water to the hydrated iodonium ion (H2OI$^+$) or hypoiodous acid (HOI). The process of electrophilic substitution in the direct labeling method involved two processes, mono and diodinated, which resulted in 2 forms. The I^+ is attached at the ortho position because it is supported by the presence of a hydroxy group (-OH) with large electron-withdrawing properties [21, 22]. Estradiol labeled with the complexes by electrophilic substitution was presented in (Figure 2).

The initial stage of radiolabeling ^{131}I-estradiol was carried out by determining the appropriate mobile phase and stationary phase for the separation of ^{131}I-estradiol from its impurities. Based on the radioiodination reaction, the radiochemical impurities are free Iodine-131 and reduced Iodine ($^{131}I_2$). The percentage of these radiochemical impurities can be determined by paper chromatography. Several eluents were tested to determine the best separation from impurities of free Iodine-131 and reduced Iodine ($^{131}I_2$). Table 1 shows five types of eluents that can separate free Iodine-131 impurities where Benzene-ethanol eluents can separate $^{131}I_2$ from ^{131}I-estradiol labeled compound, but cannot separate free Iodine-13. Whatman 1 as stationary phase and methanol:water (90:10) as mobile phase were selected based on the optimum separation, sharpness of peaks, fast elution time, and more economical raw material than TLC-SG (Figure 3).

The radiochemical purity determination of ^{131}I-estradiol was also carried out by the electrophoresis method. This method was chosen based on the chemical properties of ^{131}I-estradiol and the compounds to be separated. Electrophoresis used Whatman 1 and electrolyte buffer phosphate (0.1M, pH 7.4) for 1 h at 200 V. The results showed that free Iodine-131 with negative charge was at the anode (the right peak), while ^{131}I-estradiol was at the cathode (the left peak), and $^{131}I_2$ as the neutral charge was at the center peak, as shown in Figure 4.

The incubation time is one of the important parameters in labeling optimization, but the optimum reaction was chosen at 5 min considering that CAT can oxidize the compound to be labeled and also damage the compound. Variation of incubation was carried out at 1, 5, and 15 min and the radiochemical purity was obtained at 72.00 ± 3.71%, 97.66 ± 0.70%, and 93.27 ± 1.61% respectively. According to Fig ure 5, one-minute incubation was not sufficient to obtain the optimal labeling conditions and the optimum incubation of estradiol with Iodine-131 was at 5 min with the highest radiochemical purity of more than 90%. The presence of free radioiodide (5%) in radioiodinated radiopharmaceuticals was the most common example of radiochemical impurities.

Radioiodination of estradiol had been carried out by using CAT from 10 to 1000 μg as a mild oxidizing agent, transforming iodide (I^-) to an electropositive form of iodine (oxidative state I^+) [23, 24]. The results obtained showed that the optimum amount of CAT was 100 μg with a radiochemical purity of 96.49%. The CAT was selected at the minimum amount because of the oxidizing effect that can damage the labeled compound (Figure 6). The optimum pH for labeling using CAT was at pH below 6.5 and above 8.5 [25]. Labeling estradiol with Iodine-131 was conducted at pH 7.0 using phosphate buffer and obtained the optimum result. The pH of a labeled compound ideally should be very close to the pH of blood (7.4) intended for intravenous administration [26]. To determine the optimum results, the amount of estradiol was varied from 10-100 μg. Radiochemical purity from variation of 2, 10, 20, 50 and 100 μg estradiol was resulted in percentage of 87.39 ± 1.66%, 89.47 ± 5.45%, 95.41 ± 4.04%, 99.20 ± 0.93%, and 95.26 ± 0.354 (Figure 7). According to these data, the optimum amount of estradiol for labeling was 50 μg.

Labeled compounds obtained with high radiochemical purity also needed to be tested for storage stability. It was important to determine the period and temperature storage of the labeled compound because high specific radioactivity and high linear energy transfer of Iodine-131 resulted in a high level of free radical production. The radiolysis effect could be prevented by keeping the supplied labeled compound frozen for several days [26, 27]. The test was carried out at three different storage temperatures for 30 days (Figure 8). The results showed that ^{131}I-estradiol stored at room temperature (25 °C) until the 8th day had radiochemical purity of 97.99 ± 0.11, and ^{131}I-estradiol stored in a refrigerator (2–5 °C) and freezer (−20 °C) until the 30th day had radiochemical purity of 92.57% ± 0.84 and 84.80 ± 6.56% respectively. The highest radiochemical purity of ^{131}I-estradiol was in the refrigerator until 21 days with radiochemical purity of 97.60 ± 0.15% (stable above 95%). Research conducted by Robles et al. showed similar results that the radiolabeled Iodine-125 with biomolecules was more stable at 4 °C than -80 °C [24].

RadioHPLC testing was performed to confirm the results of the labeling optimization and determined the percentage of estradiol as a chemical impurity. The separation method was according to the Pharmacopoeia using Acetonitrile:Water (55:45) mixture solution. The initial stage was tested with standard estradiol of 2 μg/μL, and injected as much as 10 μL. The flow rate varied at 0.5; 0.75 and 1.0 μL/min and the peak of estradiol appeared at 4.88, 6.67, and 10.15 min respectively (Figure 9). When using UV at 254 nm, the peak of estradiol was obtained but the optimum ^{131}I-estradiol peak was not obtained. Furthermore, testing at 280 nm UV the optimum ^{131}I-estradiol peak was obtained as seen in Figure 9 [28]. As shown in Figure 10, radioHPLC testing was carried out with 2 detectors, a UV-Vis at 280 nm and a Radioactive detector using Gabi star. The optimum separation was obtained with a flow rate of 0.75 mL min with acetonitrile:water (55:45), showing the peak of estradiol at 6.62 min, Iodine-131 at 1.67 min, and ^{131}I-estradiol at 12.23 min. The radiochemical purity of ^{131}I-estradiol was 95.84 ± 0.896% and the results obtained were in accordance with the results from paper chromatography. The only impurities that appeared were free Iodine-131, without $^{131}I_2$ impurities. The results of radioiodination by Sallam and Mehany (2009) indicated that indirect iodination of estradiol will cause a significant increase in studied parameters when combined with a specific antibody than the direct method [29]. However, the direct method used in this experiment could be used to prepare ^{131}I-estradiol rapidly with good yield, both at carrier-added and no-carrier-added levels, and its specific activities greater than 95% have been obtained.

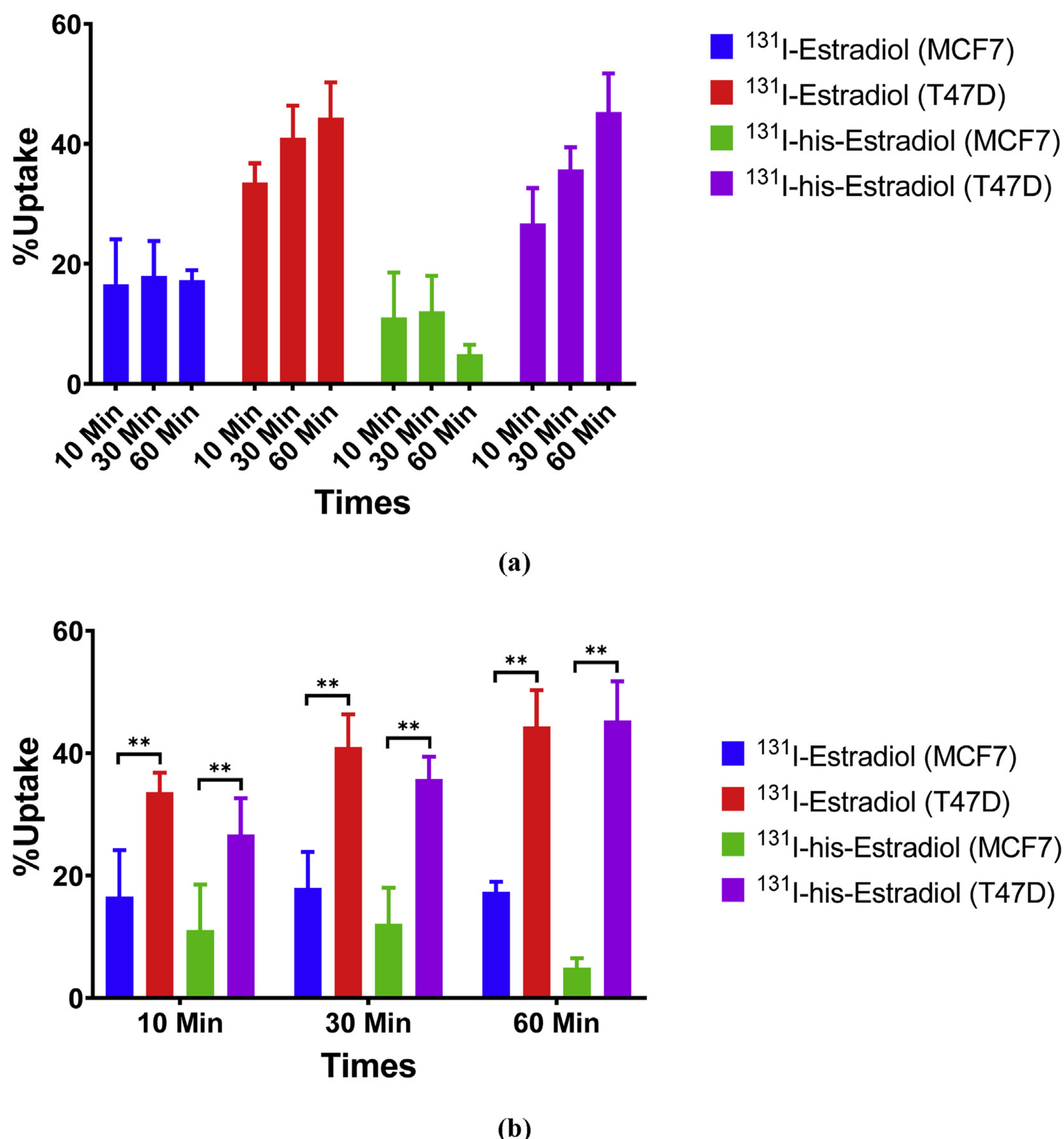

Figure 13. Cellular uptake of ^{131}I-estradiol and ^{131}I-his-estradiol at 10, 30, and 60 min. A. The uptake of ^{131}I-estradiol and ^{131}I-his-estradiol was not significantly different in the T-47D cell line ($p > 0.5$). However, The accumulation of ^{131}I-his-estradiol in the MCF7 cell line significantly decreased after 60 min incubation ($P < 0.5$). B. ** indicated $p < 0.01$.

Radioiodination of estradiol with the indirect method was carried out by labeling histamine with Iodine-131 to ^{131}I-histamine. This compound was conjugated with activated estradiol in a toluene solution containing isobutyl chloroformate and triethylamine. Conjugation was carried out in cold temperatures and the compounds formed had their radiochemical purities tested by the electrophoresis method. Radiolabeled estradiol with an indirect mechanism obtained radiochemical purity of more than 95% (Figure 11).

Cellular uptake test was carried out on estradiol labeled Iodine-131 through direct and indirect labeling with histamine and iodine-131 as control (Figures 12 and 13). Huang et al tested the cell uptake of ^{18}F-estradiol with MCF7 and T-47D which showed fast binding to the ER of 2.7% and 2.8%, respectively [8]. In this study, we used human breast cancer MCF7 and T-47D cells that has different level of estrogen receptor. The study conducted by Lostumbo et al. [30] and N. Hevir et al. [31] showed that ER level in T47-D was higher than ER level in MCF7 cell line. The cellular uptake of ^{125}I-his-estradiol has also been carried out by Susilo et al [18] with the SPA method and showed a significant binding to MCF7. In agreement with their study, this results confirmed that the higher the ER expression, the higher accumulation of ^{131}I-estradiol and ^{131}I-his-estradiol. Since this results exhibited higher accumulation of both ^{131}I-estradiol and ^{131}I-his-estradiol in T-47D than MCF7, this study was not carried out on normal cells. These results indicated the usefulness of both ^{131}I-estradiol and ^{131}I-his-estradiol to predict the ER level in breast cancer.

The important issue that hampers the clinical use to predict the ER as a part of breast cancer diagnosis is the necessity of immunohistochemistry (IHC) test as invasive methods [32]. Although IHC still used as gold standard, finding the other non-invasive method will give the second option for physician to predict the ER expression.

In this study, all requirements of ^{131}I-estradiol have been met but the short half-life of Iodine-131 is a limitation of this radioligand, so ^{131}I-estradiol must be used in RBA within 21 days. Based on the results of radiolabeling ^{131}I-estradiol, this radioligand has met the requirements of radiochemical purity (more than 95%), stable in refrigerator storage for 21 days and has the ability to uptake against MCF7 and T-47D cells. This uptake indicates that ^{131}I-estradiol and ^{131}I-his-estradiol have the

binding ability to ER after radiolabeling with Iodine-131, similar to original estradiol. In addition, ^{131}I-estradiol has the potential to be used as a labeled compound in breast cancer malignancy in the future research.

5. Conclusions

Estradiol have been successfully labeled with Iodine-131 using the direct labeling method. Determination of ^{131}I-estradiol radiochemical purity with radioHPLC was 95.84 $\pm$ 0.896% and stable for 21 days of storage in the refrigerator with radiochemical purity above 95%. ^{131}I-estradiol can be internalized in MCF7 and T-47D, as well as ^{131}I-his-estradiol until 60 min. The percentage of ^{131}I-estradiol internalization in T-47D Cell (44.34 $\pm$ 5.93%) was higher than MCF7 Cell (17.27 $\pm$ 1.71%). Furthermore, ^{131}I-estradiol can be used as a radioligand for binding assay to determine competitive binding the isolates or synthesis from natural products to estrogen receptor.

Declarations

Author contribution statement

Isti Daruwati: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Abednego Kristande Gwiharto; Ahmad Kurniawan; Isa Mahendra: Performed the experiments; Analyzed and interpreted the data.

Tri Hanggono Achmad; Mukh Syaifudin: Analyzed and interpreted the data; Wrote the paper.

Muchtaridi Muchtaridi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Applied Research Competitive Grant of Ministry of Education and Culture, Indonesia, grant number 1207/UN6.3.1/PT.00/2021 and Academic Leadership Grant Universitas Padjadjaran no.1959/UN6.3.1/PT.00/2021.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

Author thank to Nuclear Energy Research Organization-BRIN, which produced Iodine-131 by G.A. Siwwabesy Multipurpose Reactor, Serpong, Indonesia and Research Center For Radioisotope and Radiopharmaceuticals Technology, Nuclear Energy Research Organization-BRIN for radioisotope preparation. Central Laboratory and Laboratory of Cell Culture and Cytogenetics, Faculty of Medicine, Universitas Padjadjaran for MCF7 and T-47D Cell Line.

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