Quality controls of radiolabeled compounds $^{131}$I–Hippuran as PSTNT-BATAN product using electrophoresis method

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Abstract The labeled compound $^{131}$I-Hippuran has been known for a long time and was still being used by some hospitals as a medical kit for the study of dynamics kidney function. The application of Labeled compound $^{131}$I-Hippuran begins by intravenous injection to the patients, then monitor the process of biodistribution, secretion, and excretion of Labeled compound $^{131}$I-Hippuran using Renographs. The Bandung TRIGA 2000 reactor has obtained an operating license from BAPETEN in 2017, and has been operating at 800 kW to irradiate various elements including Tellurium to produce radioisotope Iodine-131. Iodine-131 was used as a radioisotope material in Labeled compound $^{131}$I-Hippuran, which was produced to fulfill the requests from An-Nur Hospital, Yogyakarta. Labeled compound $^{131}$I-Hippuran used in hospitals must meet the requirements of Indonesian Pharmacopeia, one of the parameters tested is radiochemical purity. The method used to determine the radiochemical purity is the paper electrophoresis method with phosphate buffer electrolyte pH 9, at a voltage of 300 volts for 1 hour. The radiochemical purity of $^{131}$I-Hippuran using the column chromatography method obtained yield more than 70% with radiochemical purity of 98.81 ± 0.06%. Based on these data, the radiochemical purity of the $^{131}$I-Hippuran kit has met the requirements set by Pharmacopoeia Indonesia V that is >90%.

1. Introduction

The number of patients with chronic kidney disease (CKD) in Indonesia is increasing every year. Based on Indonesia's Basic Health Research (Riskesdas 2018), the prevalence of CKD is 3.8 percent or an increase of 1.8 percent compared to 2013 [1]. Indonesian Renal Record Data (IRR) notes, patients suffering from kidney disease requiring hemodialysis or dialysis have increased by 25 thousand people in 2016 to 2017, from 52 thousand to 77 thousand patients. It is estimated that there are more than 20 thousand other kidney patients throughout Indonesia who do not have access to care [2].

The application of nuclear technology in the medical field has an important role, one of which is to detect kidney function using a compound marked $^{131}$I-Hippuran which has been known for a long time and is still used by several hospitals [3, 4, 5, 6]. The Bandung TRIGA 2000 reactor was granted an operating license in 2017 and had been operating at 800 kW to irradiate various elements, one of which is Tellurium to produce Iodine-131 radioisotope. Iodine-131 radioisotope is obtained from the tellurium metal target irradiated by neutrons in the reactor, producing a nuclear reaction: $^{129}$Te (n,$\gamma$) $^{131}$Te. The abundance of $^{131}$Te isotopes in nature (θ) is 34.49%, has a cross-section of the core reaction (σ) for the (n,$\gamma$) reaction warehouse of 0.04 and the half-life of $^{131}$Te is 30 hours. Radioisotope $^{131}$Te decays by releasing beta-negative / negatron (β-) rays by 82% to $^{131}$I, while other parts decay through an isometric transition of 12% to $^{131}$Te with a half-life of 24.8 minutes and then decays by emitting negatrons to $^{131}$I radioisotopes. Other reactions between the target $^{131}$Te and neutrons will occur such as $^{131}$Te (n,$\gamma$) $^{132}$Te, then $^{132}$Te decays by emitting negatrons to $^{131}$I, then $^{131}$I is used as a radioisotope in $^{131}$I-Hippuran labeled...
The method used in labeling hippuran compounds is through the isotope exchange method, where $^{127}$I which is stable in the otho iodo hippuric acid (OIH) compound was exchanged for a radioactive $^{131}$I radioisotope. Isotope exchange reaction is carried out in an acidic atmosphere (pH 3-4) by heating using an autoclave at 120 °C for 3 hours.

The production of the $^{131}$I-Hippuran labeled compound is carried out to supply the demand of An-Nur Hospital, Yogyakarta. Therefore, the quality of $^{131}$I-Hippuran must fulfill the requirements of Pharmacopoeia Indonesia V, Radiochemical purity >90% [7]. The method used to determine the radiochemical purity of $^{131}$I-Hippuran is to use a paper electrophoresis method with phosphate buffer electrolyte pH 9, at a voltage of 300 volts for 1 hour. The results of $^{131}$I-Hippuran purification using column chromatography method obtained >70% yield with a radiochemical purity of 98.81 ± 1.06%

The application of the $^{131}$I-Hippuran labeled compound begins by injecting the patient intravenously, then observing the process of biodynamic, secretion, and excretion of $^{131}$I-Hippuran labeled compound using Renograph. The method of detecting kidney function with the first Renograph is by injecting a radioisotope into the patient's blood vessels. Then wait for five minutes so that $^{131}$I-Hippuran was distributed to both kidneys, then radioactivity measurements were carried out on the kidneys using the Renograph. Iodine-131 has a half-life of 8 days. The half-life indicates the period of decay of the radioactive substance. National Nuclear Energy Agency (BATAN) since the 1990's developed nuclear technology for the health sector. One of them, BATAN developed a kidney function detector (Renograph) that has an advantage compared to ordinary determination tools that can be operationalized to show the analysis of the fast and accurate results in 20 minutes. Renographs has a smaller size, cheaper and easier to operate, so it will reduce the overall costs [8, 9, 10].

2. Materials and Methods

2.1. Tools
The main tool used is a process box equipped with lead (Pb), Dose Calibrator (Biodex) as a measure of radioactivity, Single Channel Analyzer (Ortec Canberra) as a chromatographic counter, a Multi-Channel Analyzer (MCA) counter to measure the purity of radionuclides and electrophoresis devices.

2.2. Materials
The main ingredients used in the manufacture of $^{131}$I-Hippuran labeled compounds were Iodine-131 (Na-$^{131}$I) radioisotope product of TRIGA 2000 Reactor, sterile aquabides (IPHA), pH Indicator universal (Merck) paper, chromatographic paper (Whatman 1), Na$_2$HPO$_4$ and Na$_2$HPO$_4$.12H$_2$O (Merck).

2.3. Research Methods
Prepared tools and reagents are also supporting materials needed for testing the quality of $^{131}$I-Hippuran solutions.

2.3.1. Electrophoresis. Determination of the quality of $^{131}$I-Hippuran labeled compound through the paper electrophoresis method which consists of Whatman 1 paper measuring 1.5 x 38 cm, each cm was numbered start from -14, -13, -12, -11, -10 to +14 as the stationary phase and phosphate buffer electrolyte pH 9 as the mobile phase. The $^{131}$I-Hippuran labeled compound was dropped at the 0 points, the left and right end of Whatman 1 paper was dipped to the electrolyte solution, and moistened with a phosphate buffer electrolyte of pH 9. The electrophoresis device was set at 300 Volts for 1 hour.

2.3.3 Physicochemical Test. Parameters tested in pharmacokinetic testing include:

1. Clarity  
   Performed visually by using bright lights and observed against a dark background.
2. Radioactive concentration test  
   Determined by dose calibrator by selecting energy (channel) according to the radioisotope to be determined.
3. pH test
3. Results and Discussion

Paper electrophoresis was a type of electrophoresis which consists of paper as a stationary phase and charged particles dissolved as mobile phase, mainly complexions. This separation occurs due to the gradation of concentration along with the separation system [11]. The movement of particles in the paper depends on the charge or valence of the solute, the cross-sectional area, the voltage used, the electrolyte concentration, ionic strength, pH, viscosity, and adsorption of the solute [12].

The physicochemical test is a determination of physical characterization, namely clarity can be done visually by using bright lights and observed against a dark background. A color check can also be visual like a clearness check, but it is not necessary with a black background. The radioactive concentration check is the amount of radioactivity per unit volume (mCi/mL) this needs to be determined for the size of the injection dose needed. The radioactivity determined by the dose calibrator by selecting energy (channel) according to the radioisotope to be determined. A chemical test that is checking the acidity of the solution (pH) can be determined with pH Indicator paper or a pH meter. Radionuclide purity check is the ratio of radionuclide activity in the form of the desired compound to the total radioactivity of the radionuclide. Radiochemical purity can be interpreted to mean there are no substances other than those listed in the formulation of the preparation, due to the causes of chemical impurity: the basic ingredients are impure, decomposition of compounds forming other compounds and contaminated in the manufacturing process [13].

Each labeled compound produced must be tested for quality and fulfill the specified requirements. In the production of 131I-Hippuran labeled compound, a radiochemical purity test was carried out on the raw material Iodine-131 [14]. The test results are shown in Figure 1.

![Figure 1. The radiochemical purity of Iodium-131](image)

Figure 1. Showed a graph of the relationship between elution distance and counts. Chromatographic elution uses the stationary phase of Whatman 1 paper and the mobile phase of methanol 75%. The test results showed that the radioisotope Iodine-131 in position +11 with a radiochemical purity of 99.90%. This shows that Iodine-131 has fulfilled the requirements as a raw material for 131I-Hippuran labeled compound production. The large radiochemical purity of Iodine-131 will produce a large radiochemical purity of 131I-Hippuran labeled compound. Before the radiochemical purity testing of 131I-Hippuran labeled...
compound, purification was carried out by the column chromatography method using dowex resin which aims to remove the Iodine ion content from $^{131}$I-Hippuran mixture. From the purification results obtained yields 60-70%, with radiochemical purity >90%.

The product $^{131}$I-Hippuran as a physical characterization of a clear solution is checked for quality by the paper electrophoresis method using the stationary phase of Whatman 1 paper and the buffer phase of pH 9. The results of the $^{131}$I-Hippuran radiochemical purity test are shown in Figure 2.

![Figure 2. The radiochemical purity of Iodium-131](image)

All batches of $^{131}$I-Hippuran results produced at PSTNT-BATAN ware shown in Table 1.

| Batch | The radiochemical purity of Iodium | Radiochemical purity of $^{131}$I-Hippuran | Pharmacopoeia Indonesia V requirement (95%) |
|-------|-----------------------------------|-----------------------------------------------|-------------------------------------------|
| 1     | 98.53%                            | 97.89 ± 2.89%                                 | √                                         |
| 2     | 99.90%                            | 99.15 ± 0.21%                                 | √                                         |
| 3     | 96.65%                            | 97.50 ± 1.10%                                 | √                                         |
| 4     | 95.60%                            | 99.73 ± 0.04%                                 | √                                         |
| 5     | 95.60%                            | 99.80 ± 0.07%                                 | √                                         |

Figure 2. Showed a graphic of the relationship between elution distance and counts. Chromatographic elution uses the stationary phase of Whatman 1 paper and the mobile phase of phosphate buffer 9. The test results show that radioisotope $^{131}$I-Hippuran is in positive position 4 with a radiochemical purity of 99.80%. Table 1. shows the results of the radiochemical purity of $^{131}$I-Hippuran from batches 1 to 5 fulfilling pharmacopoeia V requirements of >90%.

4. Conclusion
The Labelled compound $^{131}$I-Hippuran has been successfully made from batch 1 to batch 5. Radiochemical determination of $^{131}$I-Hippuran labeled compound using electrophoresis method produces radiochemical purity of 97.89% ± 2.8991; 99.15% ± 0.2051; 97.50% ± 1.0960; 99.73% ± 0.0424;
99.80% ± 0.0707. It can be concluded that 131I-Hippuran produced by PSTNT-BATAN from batch 1 to batch 5 has fulfilled the pharmacopeia V requirement of >90%.

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