Stability of $^{131}$I-Ortho-Iodo-Hippuric Acid ($^{131}$I-Hippuran) Labelled Compound Produced by CANST (Center of Applied Nuclear Science and Technology) -BATAN Bandung

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Abstract. $^{131}$I-ortho-iodo-hippuric acid ($^{131}$I-Hippuran) labelled compound has been used in nuclear medicine for renal function evaluation. The labelled compound solution administered to patients via intravenous injection. Radiation from the labelled compound tracked by gamma detector in Renograph. PSTNT has succeeded produce $^{131}$I-Hippuran to cover the demand from An Nur Urology-Specialized Hospital, Yogyakarta. $^{131}$I-Hippuran was purified by column chromatography method. HPLC analysis were performed to specified which fraction has higher radiochemical purity and will be used for patients. $^{131}$I-Hippuran stability testing needs to be done related to the decay of radioactive compounds. Three storage temperatures were prepared as follows: 4, 25, and 30°C. Radiochemical purity of each temperature determined by paper electrophoresis method on day 1, 6, 9, 13, 20, 23, and 30. The static phase used was whatman 1 paper, while mobile phase was phosphate buffer pH 9. The initial radiochemical purity is 99.52%. While on 30th day, radiochemical purity in storage temperatures of 4, 25, and 30°C are 97.04, 96.37, and 96.96% respectively. Statistical analysis using SPSS was performed to determine whether there was a significant difference in the radiochemical purity of each storage temperature. Normality and homogeneity test showed the radiochemical purity is normally distributed and has homogenous variance at three different storage temperature. Furthermore, the result of One-way ANOVA test showed that there was no significant difference of radiochemical purity in various storage temperatures. In conclusion, $^{131}$I-Hippuran labelled compound produced by CANST-BATAN Bandung is relatively stable up to 30 days and not affected by storage temperature.

Keywords: Stability testing, Labelled compound, Iodium-131 Hippuran, Radiochemical purity.

1. Introduction

Kidney is an important organ which function is excretion of the waste products of metabolism in urine [1]. Kidney, that medically called “renal”, can stop working properly for a number of reasons. People who have diabetes or high blood pressure are at greater risk. Kidney disease damages the kidney filters so that they can’t remove wastes and water. When enough of the filters are damaged, the body will fill up with excess wastes and water that would normally be removed by the healthy kidneys [2].

There are two categories of renal radiopharmaceuticals: functional agents and morphological agents. Functional agents include iodine-labeled hippuran and $^{99m}$Tc-MAG3 [3]. For many years $^{131}$I-
Hippuran has been used as a tracer to measure effective renal plasma flow (ERPF) [4]. The labelled compound solution administered to patients via intravenous injection. Radiation from the labelled compound tracked by gamma detector in Renograph.

PSTNT-BATAN have been doing research and development in the field of radioisotopes and labelled compound. 

$^{131}$I-Hippuran is one of labelled-compound which produced and has been utilized to some hospitals in Indonesia. Radioisotope iodine-131 obtained from TRIGA 2000 Bandung is used to make $^{131}$I-Hippuran labelled compound. The radioisotope is reacted with ortho-iodo-hippurate (OIH) to become the labelled compound.

![Chemical structure of $^{131}$I-Ortho-Iodo-Hippuric Acid ($^{131}$I-Hippuran) [7]](image)

As with other drug products, small-scale preparation of radiopharmaceuticals (SSRP) are expected to remain stable during storage. Although SSRPs may have extremely short shelf lives, because of the short half-lives of the labelling radionuclide, compared to other kinds of drug products, there are stability concerns due to radiation-related radiolysis. Examples of stability parameters include radiochemical identity and purity (including levels of radiochemical impurities), appearance, pH, stabilizer or preservative effectiveness and chemical purity. It is recommended that appropriate stability indicating methods that can distinguish degradation products and impurities are used. [5]

Parameters of radiopharmaceutical quality control are radionuclide purity control, radiochemical purity control, chemical purity control, biological control, and physical inspection [6]. In this stability testing, the parameters checked was radiochemical purity. Three different storage temperatures prepared were 4, 25, and 30°C. Stability testing was performed until day 30 of storage. Statistical evaluation was performed to determine whether there is a difference between the radiochemical purity of each storage temperature up to day 30.

2. Materials and Methodology

2.1 Materials

The materials used were $^{131}$I-Hippuran (PSTNT-BATAN), whatmann 1 (Whatman), buffer phosphate pH 9, pH universal (Merck, Germany)

The equipment were a set of electrophoresis instrument, refrigerator (Electrolux), incubator (Memmert) and HPLC equipped with radioactive detector GABY-star (Agilent).

HPLC analysis was performed as a qualitative analysis ortho-iodo-hippuran (OIH) already labeled by the iodine-131 radioisotope.

2.2 Radiochemical Purity Determination

Stability testing performed by preparing three storage temperature for $^{131}$I-Hippuran. Three storage temperatures were prepared as follows: 4, 25, and 30°C. Storage temperature of 4°C was made by refrigerator, while 25°C was room temperature, and 30°C was made by incubator.

The stability of the labeled compound showed by the amount of radiochemical purity. Radiochemical Purity of each temperature determined by paper electrophoresis method on day 1, 6, 9, 13, 20, 23, and 30. The static phase used was whatman 1 paper, while mobile phase was phosphate
buffer pH 9 to separate the impurities of $^{131}$I. The voltage used was 300 volts for one hour long. The chromatograms were dried in the oven at 80°C, then every 1 cm piece of paper was cut and measured using Single Channel Analayzer (SCA) with NaI(Tl) detector.

3. Result and Discussion
Ortho-iodo-hippuran (OIH) has been successfully labeled with iodine-131 radioisotope. This showed by HPLC chromatogram below.
$^{131}$I-Hippuran was purified using column chromatography method. The column was filled by dowex resin to remove I⁻ ion from labelled compound. This purification process brings out 3 (three) fractions. HPLC analysis was done to determine which fraction has the highest radiochemical purity, qualitatively. HPLC analysis was run with conditions as follows: static phase of C-18 (SGE column), mobile phase of methanol (Merck, Germany): water (40:60), flowrate of 1 ml/min, detected by UV 254nm and radioactive detector (NaI(Tl)). The result from HPLC detection of each compounds can be seen in Figure 2. OIH compound has two peaks, at 2.23’ and a small peak at 4.72’. While Na$^{131}$I time retention ($t_r$) was at 1.92’.

Figure 2 (d), chromatogram of fraction 2, showed the most similar time retention and peak with the OIH compound in Figure 2 (a) both at UV 254 nm and radioactive detector. So, fraction 2 was selected to be delivered and used for patients.

In this study, the stability of the $^{131}$I-Hippuran labelled compound was determined by the radiochemical purity. $^{131}$I-Hippuran stability testing needs to be done related to the decay of radioactive compounds. Three storage temperatures were prepared as follows: 4, 25, and 30°C. Radiochemical purity of each temperature determined by paper electrophoresis method on day 1, 6, 9, 13, 20, 23, and 30. The static phase used was whatman 1 paper, while mobile phase was phosphate buffer pH 9 to separate the impurities of $^{131}$I⁻. The voltage used was 300 volts for one hour long.

Table 1. Radiochemical Purity of $^{131}$I-Hippuran Labelled Compound

| Day | Temperature (°C) |
|-----|------------------|
|     | 4                | 25               | 30               |
| 1   | 99.52%           | 99.52%           | 99.52%           |
| 6   | 99.66%           | 99.16%           | 98.93%           |
| 9   | 99.52%           | 98.72%           | 98.43%           |
Radiochemical purity of $^{131}$I-Hippuran in 3 different storage temperatures on day 1, 6, 9, 13, 20, 23, and 30 are shown in Table 1. Statistical test was carried out to determine whether the radiochemical purity of labelled compound affected by the storage temperature. Statistic application use in this study is SPSS Statistics 25.

First, the data is tested for normality with the Shapiro-Wilk test because the data used are less than 50. The data is declared normally distributed if Sig. > 0.05. The results show $^{131}$I-Hippuran Radiochemical Purity values of 25°C and 30°C were normally distributed with a significance value of 0.476 and 0.940 (Table 2). While at 4°C $^{131}$I-Hippuran Radiochemical Purity wasn’t normally distributed, represented by significance value of 0.016.

**Table 2. Normality Test of $^{131}$I-Hippuran Radiochemical Purity**

| Tests of Normality | Kolmogorov-Smirnov a | Shapiro-Wilk |
|--------------------|----------------------|--------------|
| Storage Temperature | Statistic | df | Sig. | Statistic | df | Sig. |
| $^{131}$I-Hippuran Radiochemical Purity | 4°C | 0.274 | 7 | 0.121 | 0.759 | 7 | 0.016 |
| | 25°C | 0.192 | 8 | .200' | 0.926 | 8 | 0.476 |
| | 30°C | 0.151 | 6 | .200' | 0.978 | 6 | 0.940 |

* This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Test of Homogeneity of Variance was performed by Levene’s test to find out Homogeneity of each group. Significance value > 0.05 shows that the group has a homogeneous variant. Table 3. Shows $^{131}$I-Hippuran Radiochemical Purity significance value > 0.05, which means data is homogeneous.

**Table 3. Homogeneity Test of $^{131}$I-Hippuran Radiochemical Purity**

| Test of Homogeneity of Variance | Levene Statistic | df1 | df2 | Sig. |
|---------------------------------|-----------------|-----|-----|------|
| $^{131}$I-Hippuran Radiochemical Purity | Based on Mean | 1.694 | 2 | 18 | 0.212 |
| | Based on Median | 0.619 | 2 | 18 | 0.549 |
| | Based on Median and with adjusted df | 0.619 | 2 | 16.233 | 0.550 |
| | Based on trimmed mean | 1.654 | 2 | 18 | 0.219 |

It is known that data is normally distributed within 2 groups and isn’t normally distributed within other group, and has homogeneous variant. Furthermore, the data then analyzed by One-way ANOVA test followed by post-hoc analysis on data that doesn’t haven’t been transformed. It can be done because One-way ANOVA is still good enough to be used on data that is not normally distributed and has a homogeneous variant [13]. The results of the one-way ANOVA analysis shows that there were no significant differences between groups (Sig. > 0.05) with a significance value of 0.941 (Table 4).
Table 4. ANOVA one-way Test of $^{131}$I-Hippuran Radiochemical Purity

| $^{131}$I-Hippuran Radiochemical Purity | Sum of Squares | df | Mean Square | F     | Sig. |
|----------------------------------------|----------------|----|-------------|-------|------|
| Between Groups                         | 0.147          | 2  | 0.073       | 0.061 | 0.941|
| Within Groups                          | 21.728         | 18 | 1.207       |       |      |
| Total                                  | 21.874         | 20 |             |       |      |

Table 5. Post-hoc Test of $^{131}$I-Hippuran Radiochemical Purity

Multiple Comparisons

| (I) Storage temperature | (J) Storage temperature | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|-------------------------|-------------------------|-----------------------|------------|------|------------------------|
|                         |                         | 4                     | 25         | .15589 | .56862 .787 -1.3505 1.0387 |
|                         |                         | 30                    | .03119     | .61125 | .960 -1.2530 1.3154    |
|                         |                         | 4                     | 25         | .15589 | .56862 .787 -1.0387 1.3505 |
|                         |                         | 30                    | .18708     | .59335 | .756 -1.0595 1.4337    |
|                         |                         | 4                     | 25         | .03119 | .61125 .960 -1.3154 1.2530 |
|                         |                         | 30                    | .18708     | .59335 | .756 -1.4337 1.0595    |

According to the data that are compared to each other above, it can be stated that the radiochemical purity of each $^{131}$I-Hippuran storage temperature doesn’t significantly different. Significant differences will be indicated by an asterisk (*) after the mean difference value on the table. This shows that $^{131}$I-Hippuran labelled compound produced by CANST-BATAN Bandung is relatively stable up to 30 days and not affected by storage temperature.

4. Conclusion

CANST-BATAN has succeeded to made $^{131}$I-Hippuran labeled compound that has been used in some hospitals in Indonesia to perform renal function evaluation. This study shows the stability of the labeled compound in 3 different storage temperatures until day 30. The stability of $^{131}$I-Hippuran is represented by the radiochemical purity. According to the results of statistical test on the labelled compound radiochemical purity, $^{131}$I-Hippuran labelled compound produced by CANST-BATAN Bandung is relatively stable up to 30 days and not affected by storage temperature.

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