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Biodistribution and Imaging of The ^{99m}Tc-Glutathione Radiopharmaceutical in White Rats Induced with Cancer

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ABSTRACT

^{99m}Tc-glutathione (^{99m}Tc-GSH) is a radiopharmaceutical which is potentially used as a cancer diagnostic kit. As with other radiopharmaceuticals, before applied in humans, including in clinical trials, ^{99m}Tc-GSH needs to go through a series of preclinical trials in animal first. The preclinical trial which has been done in this study is the evaluation of the biological test on biodistribution and imaging of white rats (*Rattus norvegicus*) which had been induced with cancer. The aims of this research were to obtain data on biodistribution and to image the biodistribution of ^{99m}Tc-GSH at 1 hour, 3 hours, and 24 hours post-injection. Biodistribution results of ^{99m}Tc-GSH in the cancer at the times of 1 hour, 3 hours, and 24 hours after injection were 0.66% ID/g, 0.95% ID/g, and 0.06% ID/g, respectively. This result shows that the highest accumulation of ^{99m}Tc-GSH in cancer occur at 3 hours post-injection. This value indicates that the optimal accumulation of the ^{99m}Tc-GSH occur in this time interval. In addition, the results of imaging test also show that the accumulation capacity of ^{99m}Tc-GSH in cancer is also highest at 3 hours post-injection.

INTRODUCTION

Cancer is defined as a group of diseases characterized by uncontrolled growth and spread of abnormal cells. In the body, the cancer cells will spread to other body parts. This process can cause damages to the physiological function, including death [1]. Genetically, there are three categories of genes which play an important role in regulating the growth of cancer cells. The first category includes proto-oncogenes which regulate the life cycle of cell, cell enlargement, DNA replication, cell division, and transfer of sets of genes to daughter cells. The second category is genes which restrict growth of cancer cell or also called cancer suppressor genes. The last category is genes which regulates replication and repair of DNA. Most cancers come from a mutation in one or more categories of genes [2].

Cancer is one of the deadly diseases which have killed millions of people in the world. Based on data from Health Research Association (2007), in Indonesia, cancer reached 10.2% of the proportion of the total incidences of non-contagious diseases and became the seventh-leading cause of death from diseases [3]. In 2007 UICC (Union Internationale Contre le Cancer) reported that 8 million people worldwide died of cancer; this number comprises about 13% of the total deaths from diseases. Furthermore, this number was predicted to increase to 11.8 million deaths in 2030 [4]. The high mortality number is caused by the late cancer diagnoses where the incidence is usually discovered when the cancer has reached an advanced stage or when it displays clinical symptoms in patients. Therefore, early diagnoses are needed to reduce the mortality due to cancer.

Presently, nuclear-based imaging techniques using radiopharmaceuticals has proven its performance in cancer diagnosis. The advantages of diagnosis method using radiopharmaceuticals over conventional diagnosis method are: higher sensitivity and specificity; pain-free operation; and speed [5]. In this study, one of the potential radiopharmaceuticals for in cancer diagnosis, namely ^{99m}Tc-glutathione (^{99m}Tc-GSH), is studied.

The ^{99m}Tc-GSH radiopharmaceutical is a nuclear medical material consisting of glutathione compounds labeled with the technetium-99m radioisotope. ^{99m}Tc-GSH has been successfully synthesized by the Labelled Compound and

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Radiometry Division, Nuclear Technology Center for Materials and Radiometry-BATAN. The compound is expected to be used in nuclear medicine as a diagnostic kit for early detection of cancer. Early detections of cancer incidence are needed to determine the presence of cancer in the body in an early stage. This is very important because the therapy process is easier in the early stages of cancer than in the advanced stages.

Any radiopharmaceutical used for cancer diagnosis, including ^{99m}Tc-GSH, should optimally accumulate in target organ, namely the cancer, so the imaging process can be applied to obtain the visual data of cancer. In addition, ^{99m}Tc-GSH must also be non-toxic, non-pyrogenic, and not harmful to the body. Therefore, to ensure fulfillment of the above requirements, before it can be applied as a diagnostic kit, ^{99m}Tc-GSH must pass biological evaluation stages (preclinical trials) first.

One of the preclinical trials is the biodistribution test. This test aims to determine the distribution of the ^{99m}Tc-GSH in several vital organs when injected into the body. It is very important in biodistribution test to observe the accumulation of ^{99m}Tc-GSH in target organs, especially the cancer. The value of ^{99m}Tc-GSH accumulation in target organs is important for the success of the diagnostics. The accumulation of a radiopharmaceutical should also be visualized in the process of imaging. Therefore, the purpose of this study is to determine the biodistribution and imaging ^{99m}Tc-GSH in the body of white rats (*Rattus norvegicus*) induced with cancer. The results from this study are expected to provide recommendations to the clinical trials in the field of nuclear medicine for cancer diagnosis.

EXPERIMENTAL METHODS

Materials and equipment

The materials used in this study were ^{99m}Tc-GSH radiopharmaceutical, DMBA (7.12 dimethyl benz(a)antracene) produced by Aldrich, 70% alcohol, corn oil, parchment paper, and white rats (*Rattus norvegicus*) of *Sprague Dawley* (SD) strain, aged 4 weeks. Equipment used were animal scanner (from Berthoid), feeding stick, Pb containers, conical tubes, single channel analyzer (SCA) (Schlumberger), syringe, vortex (Retsch), analytical scale (Mettler), dose calibrator, and dissecting instruments.

Carcinogen preparation

Carcinogen preparation was done by dissolving the DMBA carcinogen powder to the corn oil solution according to the required volume. The solution was stirred for 15 minutes until it became homogeneous.

Cancer induction in white rats

Induction of cancer was performed in female rats with DMBA. The DMBA was administered twice a week for 8 weeks at the dose of 20 mg/kg body weight. DMBA was given orally in a volume (ml) which was calculated to fit the mouth to the esophagus. Further, the rats which had been induced with cancer were maintained for 4-6 months. The DMBA dose calculation followed the following equation [6].

$$\text{DMBA Volume} = \frac{0.02\text{mg/g x body weight of rat}}{4\text{ mg/ml}} \quad (1)$$

Cancer nodule palpation

Palpation of cancer nodules in rats which had been administered with DMBA was conducted to detect cancerous tissue growth. Palpation was performed in all rats 1-2 times a week for 4-6 months. The palpation was carried out by pushing or feeling the entire body of the rats, and if any nodules (cancer) were found, the rats will be recorded as rats with cancer.

Biodistribution test

Biodistribution test in rats induced with cancer was carried out by injecting 200 μCi of ^{99m}Tc-GSH via the tail vein (intravenous). The observation was conducted at 1 hour, 3 hours, and 24 hours after injection by dissecting the rats. The cancerous lumps, muscles, hearst, small intestines, livers, spleens, kidneys, lungs, stomaches, and blood tissues were collected. All organs were weighed, then counted with SCA to obtain the accumulation of counts per gram organ. Counted values were processed to obtain the organ's percentage injected dose per gram of tissue weight (% ID/g), using the following formula, to obtain the accumulation capacity in each organ [7].

$$\%\,\text{ID/g} = \frac{\text{counts per gram organ}}{\text{counts dose given}} \text{ x } 100\% \quad (2)$$

Imaging test

Imaging tests were done on rats induced with cancer. Rats were intravenously injected with the ^{99m}Tc-GSH via the tail vein at dose of 2 mCi. The imaging process was performed at 1 hour, 3 hours, and 24 hours post-injection, on different rats, using an animal scanner.

Data analysis

All parameters in this study were observed as triples. The data were analyzed using a quantitative ANOVA (Analysis of Variance) and DMRT (Duncan Multiple Range Test). Significance level used is 5% to determine whether there is any difference in accumulation capacity of the rat organs, while the qualitative data were analyzed by descriptive statistics.

RESULTS AND DISCUSSION

The ^{99m}Tc-GSH radiopharmaceutical injected via the tail vein was distributed through the body by the bloodstream. The distribution, accumulation, and ellimination of a radiopharmaceutical is highly dependent on the entry path, the chemical form, and metabolism in the body. The accumulation value (expressed in % ID/g) of the organ is important in determining the success of the diagnosis method. The accumulated value can also be used to consider the safety of a radiopharmaceutical for the body.

On the physico-chemical properties, the ^{99m}Tc-GSH used has a radiochemical purity of 99.54 ± 0.01% and is stable for up to 5 hours at room temperature and at 4^{o}C. Those properties are important for the success of the diagnosis process. The high radiochemical purity of the ^{99m}Tc-GSH means that there was less impurities in stocks. Hence it made the ^{99m}Tc-GSH relatively stable, especially from its chemical bonds so that the accuracy of the data collection can be recognized [8].

The ^{99m}Tc-GSH application in this study was carried out by injection through the tail vein. This was aimed at accelerating the distribution of the radiopharmaceutical to the organs as it flows with the blood. It also has the benefit of reducing the residence time of the radiopharmaceutical in the body but still within the optimal duration for the diagnosis. Some studies have been conducted regarding the radiopharmaceutical biodistribution via tail vein injection process. Sumpena (2008) and Sugiharti (2009) examined the biodistribution of ^{99m}Tc-CTMP [9] and ^{99m}Tc-Human Serum Albumin [10], respectively. The biodistribution of ^{99m}Tc-GSH is shown in Fig. 1.

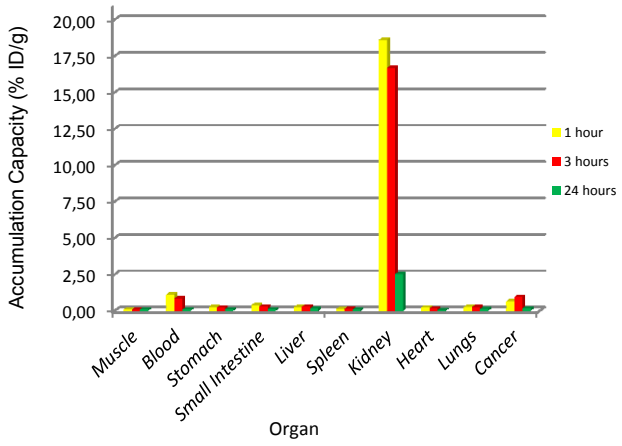

Fig. 1. Biodistribution of ^{99m}Tc-GSH in rats with cancer at 1 hour, 3 hours, and 24 hours post injection.

Biodistribution results (Fig. 1) show that the average value of % ID/g was different for different organ and different time intervals. In muscle organ the average % ID/g of ^{99m}Tc-GSH at 1 hour, 3 hours, and 24 hours after injection were 0.10, 0.08, and 0.01% ID /g respectively. There was a decrease of ^{99m}Tc-GSH in muscle organ during the time interval to 24 hours.

The decrease in accumulation value over time also happened to almost all other organs. The accumulation value for each organ after from 1 hour, 3 hours, and 24 hours were as follows: in the blood (1.12, 0.85, and 0.03% ID/g respectively), stomach (0.26, 0.22, and 0.01% ID/g), small intestine (0.38, 0.29, and 0.02% ID/g), spleen (0.13, 0.13, and 0.05% ID/g), kidney (18.61, 16.68, and 7.19% ID/g), heart (0.24, 0.17%, and 0.01% ID/g), and lung (0.27, 0.25, and 0.04% ID/g). An exception was the liver, for which there was a slight increase in the accumulation of the ^{99m}Tc-GSH at 3 hours post-injection in the amount of 0.02% ID/g.

The increased ^{99m}Tc-GSH accumulation in the liver may be caused by the wider availability of glutathione compounds in the liver compared to the other organs [11]. However, this increase is relatively small, so its impact is not significant, and at 24 hours, the ^{99m}Tc-GSH accumulation has decreased as in other organs. Sumpena (2010) also reported a decline of ^{99m}Tc-GSH accumulation over time in most organs in normal mice [12].

Overall, all organs showed decreasing ^{99m}Tc-GSH accumulation over time, and accumulation values in those organs show no significant difference except for the blood, kidneys, and cancer. Accumulation in the stomach indicates a Tc-complex decomposition in biodistribution test [13].

The decomposition of Tc-complex is closely related to the physiological function of the stomach for digesting food. In hepatobiliary organs (small intestine, spleen, and liver) the accumulation of the ^{99m}Tc-glutathione was relatively low (<0.5% ID/g). This value showed that the uptake of these organs was low so ^{99m}Tc-GSH is safe for use.

The decrease in the accumulation of ^{99m}Tc-GSH in all organs over time indicates that the clearance by each organs had occurred [14]. This is supported by the high value of % ID/g in the kidneys compared to in the other organs (Fig. 1). This situation is in line with one of the functions of kidneys, namely accommodating wastes from all cellular metabolism and excreting them through urine. The metabolic wastes are brought by the blood flow through kidney glomerulus where those wastes are filtered [15]. The clearance of the ^{99m}Tc-GSH from blood follows the same pattern with the clearance by the kidneys. From these results it can be said that the clearance of the ^{99m}Tc-GSH from the body went well because it did not accumulate in any tissue for too long; thus, it can be said that this result indicates that ^{99m}Tc-GSH is safe for the body.

Long term accumulation of a radiopharmaceutical in specific organs is very dangerous and should be avoided, as the emitted radiation of the radiopharmaceutical can damage organs. The damage potentially include various mutations and chromosome aberrations [16].

Cancer tissues, which are the main purpose of the diagnosis, accumulated ^{99m}Tc-GSH to relatively higher concentrations compared to other organs except blood and kidneys at 1 hour, 3 hours, and 24 hours with values of 0.66% ID/g, 0, 95% ID/g, and 0.06% ID/g, respectively. The high value of accumulation in the cancer is related to the function of the glutathione compound in the body. Naturally, a certain level of glutathione can be produced by body cells. However, infections or inflammations in an organ will increase glutathione levels because of the function of glutathione in the formation of antibodies in fighting infection or inflammation [17]. Therefore, ^{99m}Tc-GSH in the blood will go to the cancer as a target organ (abnormal organ). When reaching the cancer, ^{99m}Tc-GSH will be uptaken and then accumulated in those organs.

The data also shows that the highest accumulation value of ^{99m}Tc-GSH occurred at 3 hours post-injection. It can be concluded that the optimal accumulation of ^{99m}Tc-GSH for cancer diagnosis occurs at 3 hours post-injection.

A previous study conducted by Ralna (2010) on the ^{125}I-Nimotuzumab radiopharmaceutical for brain cancer therapy found that the highest accumulation occurs 24 hours post-injection at 0.13% ID/g. This value is still low compared with the accumulation of ^{99m}Tc-GSH at 3 hours post-injection which is 0.95% ID/g [18].

The high accumulation of the ^{99m}Tc-GSH in cancer at 3 hours post-injection is also supported by the ratio of cancer and blood accumulation. The ratios of % ID/g between cancer and blood at 1 hour, 3 hours, and 24 hours are 0.6, 7.4, and 2.0, respectively (Fig. 2). From these data it is observed that the highest ratio occurs at the 3 hours post-injection ^{99m}Tc-GSH.

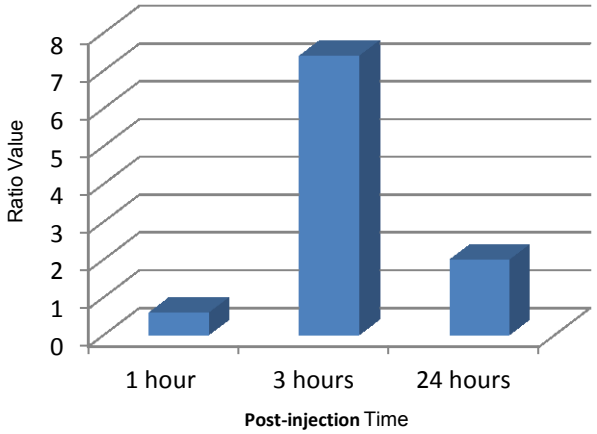

Fig. 2. The ratio of % ID/g between cancer and blood.

The cancer-% ID/g-to-blood-% ID/g ratio was highest at 3 hours post-injection. It indicated that the ^{99m}Tc-GSH was uptaken very well by the cancer at this time. The result also agrees with the value of the accumulation of ^{99m}Tc-GSH at 3 hours post-injection from the biodistribution test. The ratio may also indicate that at 3 hours post injection ^{99m}Tc-GSH has undergone the process of clearance by the blood. The higher ratio of radiopharmaceutical which is distributed to target organ to which is distributed to other organs will generate better images and facilitate more accurate diagnoses of the disease [19].

At the 24 hours post-injection the ratio of blood and cancer significantly decreased. It indicates that the uptake capacity of ^{99m}Tc-GSH by cancer has declined. ANOVA and DMRT statistical analysis show that the accumulation value of the ^{99m}Tc-GSH in rat organs show no significant difference from each other, except for the kidneys. This situation occured because accumulation values in the kidneys are much different from accumulation values in other organs. In the kidneys, accumulation values were significantly different (α = 0.05) from accumulations in other organs.

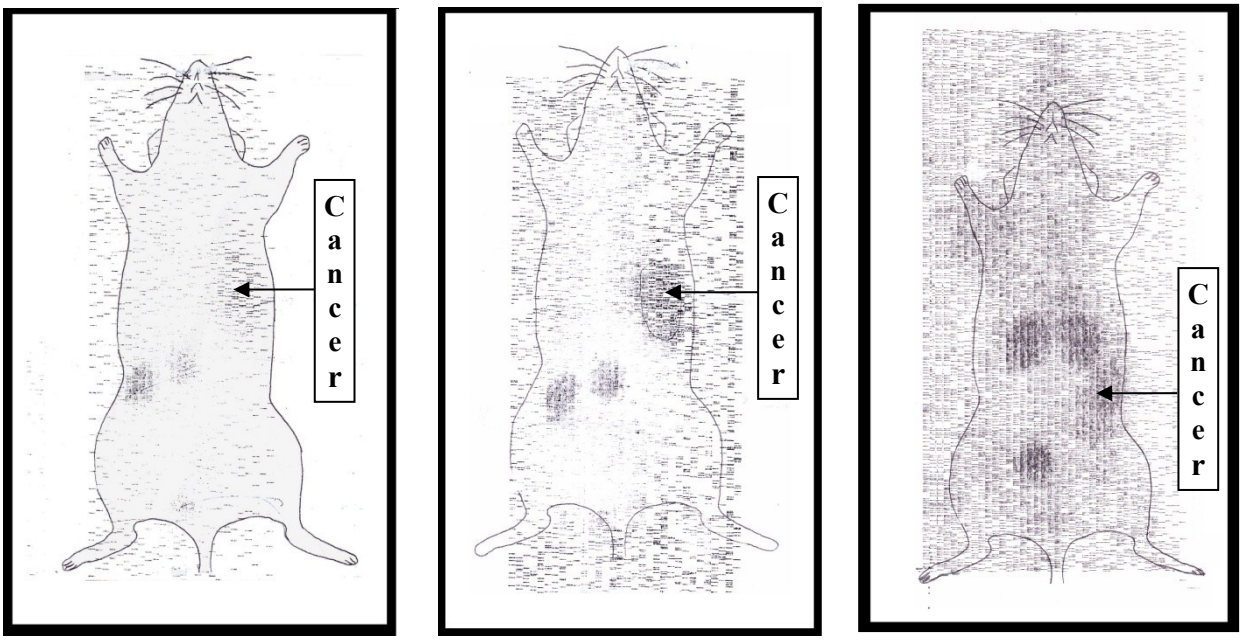

Fig. 3. Imaging of ^{99m}Tc-GSH in rats with cancer at 1 hour (a), 3 hours (b), and 24 hours (c) post injection.

Imaging test results indicate that the accumulations of ^{99m}Tc-GSH in cancer at 1 hour, 3 hours, and 24 hours post-injection are different. Figure 3 shows that the highest accumulation of ^{99m}Tc-GSH occur at 3 hours post-injection. This can be seen from the results of imaging test in which the color of cancer was darkest at 3 hours post-injection.

These imaging test results are in accordance with the biodistribution test results in which the highest accumulation of ^{99m}Tc-GSH occurred at 3 hours post-injection. At 1 hour post-injection (Fig. 3a) cancer color is not as dark as at 3 hours post-injection (Fig. 3b). This is probably because the ^{99m}Tc-GSH is still experiencing biodistibution in blood, or it may also mean that ^{99m}Tc-GSH has just started to enter the cancer organ. Further, at 24 hours post-injection (Fig. 3c), the image has become less dark than at 3 hours. This may be caused by two factors. First, the ^{99m}Tc-GSH accumulating in the cancer may have undergone a clearance. Second, it may also be caused by the decay of technetium-99m bound to glutathione group. Technetium-99m has a half-life of 6 hours [20], which means that every 6 hours the radioactivity of this compound halves. As a result, 24 hours after injection, the ^{99m}Tc-GSH radioactivity measured during imaging has decreased by over 90%.

The basic principle of imaging test is detection and quantification of the activity of a radiopharmaceutical followed by visual display of the results. In the resulting image, accumulation of the radiopharmaceutical can be observed from the indicated level of darkness of a particular organ. Darker parts indicate radiopharmaceutical accumulation as measured by the animal scanner. In this case, the presence of accumulation of dark part in a particular organ may signify that there is a growing cancer.

The higher the accumulation level of a radiopharmaceutical in an organ, the easier it becomes to create images of the organ with an animal scanner or a gamma camera [21]. However, this principle is difficult to apply to observing kidneys as they are basically always accumulating higher quantities of radiopharmaceutical than other organs. This is caused by the function of kidneys as excretory organs. Therefore, diagnosis of kidney cancer is usually conducted with a different, special method.

CONCLUSION

Based on the test results it can be concluded that ^{99m}Tc-GSH effectively reaches the target organ (cancer) at 3 hours post-injection with a concentration of 0.95% ID/g. This data agrees with the imaging test results which also showed the highest accumulation of ^{99m}Tc-GSH at 3 hours post-injection. In addition, ^{99m}Tc-GSH also showed high values of renal and blood clearance which indicates that it is relatively safe for the body. From these results, it can be concluded that ^{99m}Tc-GSH is ready to proceed to clinical trials.

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REFERENCES

1. N.L. Lubis and Hasnida, Social Support for Cancer Patients: Is it Necessary, USU Press, Medan (2009) 1.
2. R. Sofyan, Role of Nuclear Techniques in Biochemistry and Finding New Breakthrough in the Health Sector, Inauguration Speech Expert Researcher at October 24, 2002, Bandung (2002) 8.
3. Anonymous, Ministry of Health, Health Research Association: Woman is Most-group Esophageal Cancer. http://www.infodokterku. com/ component/ content/ article/ 25-data/ data-kesehatan/ 145 – data – riskesdas – perempuan-merupakan – kelompok- yang- paling- banyak-terserang-kanker. Retrieved in October (2011).
4. Anonymous, Tackling Cancer Control Worldwide, Report from the 2008 World Cancer Congress. http://www.clinical correlations.org/?p=989. Retrieved in October (2008).
5. A. Owunmane, Patel and M. Sadek, The Handbook of Radiopharceuticals, 1st Ed, Chapman and Hall Medical, London. (1995) 59.
6. Anonymous, Procedure: Antikarsinogenesis in Vivo Test, Cancer Chemoprevention Research Center, Faculty of Pharmacy UGM, Yogyakarta (2009) 3.
7. Anonymous, Work Instruction Test Radiopharmaceutical, Nuclear Technology Center for Materials and Radiometry-BATAN, Serpong (2005) 6.
8. N. Zainuddin, E. Isabela, Iswahyudi and P.P. Kania, Journal of Nuclear Science and Technology **13** (2012) 9.
9. Y. Sumpena, R.J. Sugiharti, I. Halimah, Misyetti and R. Sofyan, *Biolagical Evaluation of ^{99m}Tc-CTMP Used for Bone Imaging in Animal Model,* Proceedings of the National Seminar on Safety, Healthy and Enviromental (2008) 3.
10. R.J. Sugiharti, I. Halimah, P.P. Kania and N. Kartiny, *The Use of the Radiopharmaceutical ^{99m}Tc-Human Serum Albumin Nanosfer for Imaging and Detection of Bone Marrow Inflammation in Animal Testing,* Proceedings of the National Seminar on Safety Health and Environment V, (2009) 4.
11. Sugiyatna, Biomedical Journals **1** (2007) 48.
12. Y. Sumpena, I. Halimah, Iswahyudi, P.P. Kania and N. Zainuddin, Biodistribution of Radiopharmaceutical ^{99m}Tc-glutathione in White Mice (Mus musculus) Normal and Infected Bacteria, Research Reports, Nuclear Technology Center for Materials and Radiometry, National Nuclear Energy Agency, Bandung (2010) 3.
13. R.J. Sugiharti, Y. Sumpena and Misyetti, Journal of Nuclear Science and Technology Indonesia **10** (2009) 93.
14. H. Xie, A. Charlene, H. Mary, M.L. John and A. Walter, The Journal of Pharmacology and Experimental Therapeutics **308** (2003) 1073.
15. Anonymous, Pharmacocinetics in Patient with Renal Function Impared, Guidance for Industry, US Department of Health and Human Services (1998).
16. E.A. Rourke, S.L. Mirtha, M.M. Claudia, E.S. Michael, J.E. Carol, A. Thomas, L.B. Melissa and A.E. Randa, Cancers Journal **2** (2010) 420.
17. F.J.T. Staal, Eur. J. Clin. Invest. **28** (1998) 195.
18. R. Ralna, M. Ramli, A.H. Gunawan, A. Mutalib and A. Subarnas, Radioisotope and Radiopharmaceutical Journal **13** (2010) 1.
19. P.P. Kania, Y. Sumpena, Iswahyudi, Misyetti, And T.H. Ambarwibawa, Biodistribution and ^{99m}Tc-CTMP Animal Experiment Laboratory Scale and Scale Applications with Surgical Techniques and Scanning, Research Reports, Nuclear Technology Center for Materials and Radiometry, National Nuclear Energy Agency, Bandung (2011) 3.
20. D.B. Panular, N. Muhammad and S. Evi, Periodic Journal of Physics **7** (2004) 97.
21. G.B. Saha, Fundamentals of Nuclear Pharmacy. 5th Edition, Springer, New York (2004) 129.