

# Physicochemical and Biological Analysis of $^{99m}\text{Tc}$ -Glutathione Radiopharmaceuticals

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## ABSTRACT

$^{99m}\text{Tc}$ -glutathione ( $^{99m}\text{Tc}$ -GSH) radiopharmaceutical is available in the GSH lyophilized-kit in which ready to use directly after adding  $^{99m}\text{Tc}$  radionuclide. In nuclear medicine,  $^{99m}\text{Tc}$ -GSH diagnostic kit is a radiopharmaceutical commonly utilized for cancer diagnoses using imaging method. This paper described the physicochemical and biological characteristics as well as the quality of  $^{99m}\text{Tc}$ -GSH diagnostic kit prepared from the GSH lyophilized-kit. The radiochemical purity was determined with thin layer chromatography (TLC) method, performed on a TLC-SG stationary phase with a mobile phase of a dried acetone and 0,9% of NaCl solution. Studies on the effect of volume and radioactivity of  $\text{Na}^{99m}\text{TcO}_4$  solution to the radiochemical purity of  $^{99m}\text{Tc}$ -GSH were carried out. The sterility of GSH-lyophilized kit and toxicity of  $^{99m}\text{Tc}$ -GSH were also investigated. The stability test on GSH lyophilized kit and  $^{99m}\text{Tc}$ -GSH in several storage conditions, as well as the plasma stability of  $^{99m}\text{Tc}$ -GSH was performed. The analysis result showed that the GSH lyophilized-kit was sterile; the  $^{99m}\text{Tc}$ -GSH was non toxic with  $99.54 \pm 0.01\%$  of radiochemical purity and remained stable 5 hours either at room temperature or  $4^\circ\text{C}$ . The volume more than 4 mL of  $\text{Na}^{99m}\text{TcO}_4$  solution on the labeling of GSH could decreased the  $^{99m}\text{Tc}$ -GSH radiochemical purity, while the radioactivity more than 20 mCi in 7 mL of volume extended the incubation time. *In-vitro* stability test of  $^{99m}\text{Tc}$ -GSH in plasma showed that in the two hours of storage, the radiochemical purity decreased to  $51.84 \pm 2.52\%$ , and until 5 hours of storage it did not change significantly. From the result, it can be concluded that the GSH lyophilized-kit was remained stable after 13 month of storage either at room temperature or at  $4^\circ\text{C}$  with 99% of  $^{99m}\text{Tc}$ -GSH radiochemical purity.

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## INTRODUCTION

In Indonesia, cancer is the third leading cause of death after heart disease and infections [1]. Therefore, early detection is very important to enable early stage treatments which are relatively easier than late stage treatments. Nuclear medicine plays an important role in early-stage cancer detection. Since 2009, BATAN-Bandung has conducted research to develop a  $^{99m}\text{Tc}$ -glutathione ( $^{99m}\text{Tc}$ -GSH) radiopharmaceutical which is expected to be used as a diagnostic kit for early detection of cancer [2] in nuclear medicine.

Glutathione with sulfhydryl groups (-SH) on cysteinyl will react with  $^{99m}\text{Tc}$ , formed complex containing tetravalent or pentavalent technetium coordinated with two thiol and nitrogen atoms of two GSH ligand and an apical oxo group [3]. This radiopharmaceutical is selected for the

diagnosis of cancer, particularly head and neck cancer [2].

$^{99m}\text{Tc}$ -GSH is a chelate complex with the radionuclide  $^{99m}\text{Tc}$  as the central atom [4,5]. The radiopharmaceutical cold kits are available in the form of either dry or liquid kits which will be ready for use after reconstitution with  $^{99m}\text{Tc}$ -pertechnetate.

Two of the conditions which must be met by a radiopharmaceutical diagnostic kit are as follows. First, the radiopharmaceutical should accumulate at the target organ for an optimal duration for imaging using gamma camera. Second, its properties must not be changed by the labeling process with the  $^{99m}\text{Tc}$  radionuclide. To ensure those conditions, the radiopharmaceutical must meet the quality requirement.

Analysis of radiochemical purity of the radiopharmaceutical is necessary to ensure that it has a high purity. Therefore, it will accumulate mainly in target organs and only minimally accumulates in unintended organs. This paper

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describes the physicochemical and biological evaluation of the lyophilized GSH kit product here, which includes the following evaluations: (1) evaluation of the effects of the volume of sodium pertechnetate which is added to the lyophilized GSH kit; (2) evaluation of the effects of the radioactivity of the sodium pertechnetate; (3) analysis of the radiochemical purity of  $^{99m}\text{Tc}$ -GSH; (4) determination of the plasmatic stability of  $^{99m}\text{Tc}$ -GSH in vitro; (5) analysis of the stability of  $^{99m}\text{Tc}$ -GSH and lyophilized GSH kit in a variety of storage conditions; (6) toxicity test of  $^{99m}\text{Tc}$ -GSH in animals; and (7) sterility test of lyophilized GSH kit.

The physicochemical and biological characteristics and quality data of  $^{99m}\text{Tc}$ -glutathione obtained in this research are useful for supporting the utilization of  $^{99m}\text{Tc}$ -GSH for cancer diagnosis.

## EXPERIMENTAL METHODS

The materials used include: L-reduced glutathione (GSH) produced by Aldrich;  $\text{Na}^{99m}\text{TcO}_4$  solution obtained using  $^{99}\text{Mo}$ - $^{99m}\text{Tc}$  generator produced by Batan Technology; *aqua bide* (double-distilled water) and NaCl physiological solution for sterile injection produced by IPHA Laboratories;  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , acetone, sodium hydroxide, hydrochloric acid and other reagents of analytical grade from E. Merck; thin-layer chromatography silica gel (TLC-SG) plates from E. Merck; and disposable syringe (sizes 1, 2.5, 5 mL) from Terumo. Test animals used were 3-3,5 months old white mice of the Swiss Webster strain.

The equipment were used include Mettler-Toledo analytical balances, a Victoreen dose calibrator from Victoreen, a Retsch vortex mixer, a Memmert oven, a Denver Instrument pH-meter, a Schlumberger single-channel Analyzer, a Labconco freeze-dryer, laminar air flow, a Heraeus incubator, and a set of tools for ascending chromatography.

The rest of this section details the steps followed in this work. The steps are as follows.

### Preparation of glutathione (GSH) dry kit radiopharmaceutical

The preparation of dry kit was done using lyophilization, also known as freeze-drying. Vials containing 20 mg of GSH and 0,3 mg  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  with initial volumes of 1,3 mL and pH of 7-7,5 were prepared [6]. Sterilization was done by filtering the solution using bacterial filter (0.22  $\mu\text{m}$ ), into sterile

vials and then lyophilized for 24 hours in a freeze-dryer. The lyophilized kits were then vacuum-tested manually and their dryness was observed visually.

### Sterility test for lyophilized GSH kit

The sterility of the lyophilized GSH kits was determined by the method described in Pharmacopoeia Indonesia [7]. In this test, two kinds of media, liquid thioglycolate and nutrient agar, were used, and the test was conducted aseptically under a laminar air flow (LAF).

The lyophilized GSH kit was reconstituted with 1 mL of sterile physiological saline (0.9%) solution, and then shaken until homogeneous. The radiopharmaceutical was then smeared into the surface of each medium using needle loops. Then, the media was placed in an incubator with a temperature of 37°C and was monitored for 7-14 days for the possibility of the growth of bacteria or mold.

### Preparation of radiopharmaceutical $^{99m}\text{Tc}$ -GSH

$^{99m}\text{Tc}$ -GSH radiopharmaceutical was obtained by adding 2 mL  $\text{Na}^{99m}\text{TcO}_4$  ( $\approx$  10-20 mCi) into the lyophilized GSH kit. The kit was then shaken until homogeneous. The resulting preparation was then ready for analysis.

### Radiochemical purity determination of $^{99m}\text{Tc}$ -GSH

The radiochemical purity of  $^{99m}\text{Tc}$ -GSH was determined by ascending thin-layer chromatography method using 1×10 cm TLC-SG as the stationary phase. The TLC-SG plates were marked at each one cm from -1 to 8 as the stationary phase. As the mobile phase, two kinds of solvents were used, i.e, dried acetone and physiological saline solution (0,9%) [6].  $^{99m}\text{Tc}$ -GSH solution was spotted on a TLC-SG at zero point and elution was carried up to the limit at mark 8. Chromatograms were dried in the oven, wrapped in tape to avoid the release of powdered silica gel, then cut into 1 cm long pieces, and each piece was counted using a single channel counter with NaI(Tl) detector.

### Stability test of the lyophilized GSH kits

Lyophilized GSH kits were treated in two different storage conditions, at room temperature of

25 ± 1°C and at of 4 ± 1°C. The stability test of the lyophilized GSH kit was conducted every month by determining the radiochemical purity after labeling the kit with 10-20 mCi/ml of <sup>99m</sup>Tc.

### Toxicity testing of <sup>99m</sup>Tc-GSH

The toxicity test of <sup>99m</sup>Tc-GSH was performed using the methods described in the Pharmacopoeia Indonesia [7]. Large doses of 100 times human doses per unit body weight were used in this experiment [8]. Based on the calculations, 0.5 mL of <sup>99m</sup>Tc-GSH with an activity of 860 µCi was injected intravenously to each of five mice. The mice were 3 to 3,5 months of age. They were then observed for 48 hours after injection. If the mice stay alive after 48 hours and show no toxicity symptoms, it would indicate the absence of toxicity effects from <sup>99m</sup>Tc-GSH.

### The influence of Na<sup>99m</sup>TcO<sub>4</sub> solution volume to the radiochemical purity of <sup>99m</sup>Tc-GSH

The test was performed by addition of varying volumes (1, 2, 4, 6, 7, 8, and 10 mL) of Na<sup>99m</sup>TcO<sub>4</sub> solution of a constant activity (≈ 10 mCi) into lyophilized GSH kits. After shaken for brief time, the radiochemical purity was determined by thin layer chromatography method.

### The influence of the radioactivity Na<sup>99m</sup>TcO<sub>4</sub> to the radiochemical purity of <sup>99m</sup>Tc-GSH

A fixed volume of 2 mL of Na<sup>99m</sup>TcO<sub>4</sub> with varying activities (5, 10, 20, 30 and 40 mCi) was added into lyophilized GSH kits. After shaken for a few moments, the radiochemical purity was determined at 0, ½, 1, 2, 3, 4 and 5 hours with chromatographic methods.

Afterward, the above test was repeated with a larger volume (7 mL) of Na<sup>99m</sup>TcO<sub>4</sub>, also with varying activities (10, 20, 30, 40, 50 and 70 mCi). The resulting data is needed to determine whether the radiopharmaceutical could be used in multi-dose or single dose.

### <sup>99m</sup>Tc-GSH stability on storage

The stability of <sup>99m</sup>Tc-GSH after labeling was tested at two different temperatures, temperature of 25 ± 1°C and at of 4 ± 1°C. The radiochemical purity was observed at certain times (0, ½, 1, 2, 3, 4 and 5 hours). Determination of radiochemical purity was conducted by thin layer chromatography.

### Plasmatic stability of <sup>99m</sup>Tc-GSH

The stability of <sup>99m</sup>Tc-GSH in human blood plasma was determined by adding 250 µL of <sup>99m</sup>Tc-GSH to vials containing 750 µL of human blood plasma (human plasma). The plasma was then stirred with a vortex mixer and placed in a 37°C incubator. After mixing, radiochemical purity was determined at certain times (½, 1, 2, 3, 4 and 5 hours) with TLC method. For comparison, the stability test was also done using phosphate buffer instead of blood plasma.

## RESULTS AND DISCUSSION

The <sup>99m</sup>Tc-GSH radiopharmaceutical for the diagnosis of cancer was made in kit form, based on a formula derived in a previous study [6]. However, it must be noted here that the lyophilized GSH kit itself does not contain any radioactive substance. Therefore, it has to be mixed with Na<sup>99m</sup>TcO<sub>4</sub> solution to provide the <sup>99m</sup>Tc-GSH radiopharmaceutical. It is mandatory to perform physicochemical and biological analysis to ensure that the lyophilized GSH kits meet the quality requirements and are fit for clinical uses.

Visual observation showed that the lyophilized GSH kit was in the form of an odorless white solid which resembled cotton. <sup>99m</sup>Tc-GSH must be free of microorganisms such as bacteria or fungi which can cause infections to patients. To obtain sterile <sup>99m</sup>Tc-GSH, both the GSH dry kit and the <sup>99m</sup>Tc must be sterile, and the addition of <sup>99m</sup>Tc to the kit has to be performed aseptically. In the sterility testing, the addition process was performed in a Laminar Air Flow (LAF) cabinet. Solid nutrient agar was used to determine the presence of bacteria, while thioglycolate liquid medium was used to detect fungi. The sterility testing was performed with three repetitions of the GSH dry kits drawn at random. Visually, the tests showed no growths, both of bacteria and fungi, in any media after a seven-day incubation period. This means that the GSH dry kits were sterile.

The average of radiochemical purity of 15 <sup>99m</sup>Tc-GSH replicates was 99.54 ± 0.01%, with very low radiochemical impurities, in the form of free pertechnetate (<sup>99m</sup>TcO<sub>4</sub><sup>-</sup>) and reduced-<sup>99m</sup>Tc (<sup>99m</sup>TcO<sub>2</sub>) were 0.116 ± 0.01% and 0.30 ± 0.01% respectively. The radiochemical purity of <sup>99m</sup>Tc-GSH obtained from this experiment, shown in Fig. 1, are similar to the values obtained in previous studies, with a radiochemical purity of greater than 95% [6]. The radiochemical purity of <sup>99m</sup>Tc-GSH found in this study meets the requirements for the radiopharmaceutical, which is ≥ 90% [9], and shows

good reproducibility, as seen from the small standard deviation.

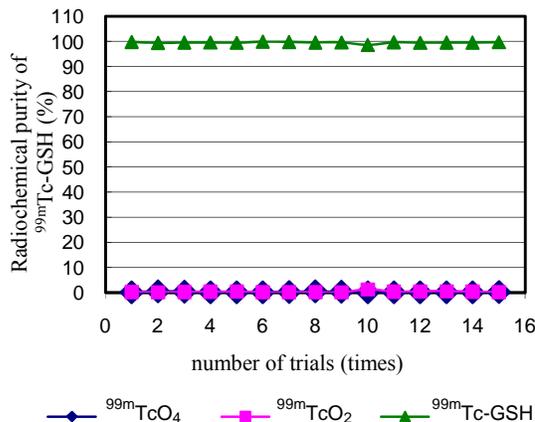


Fig. 1. Radiochemical purity of <sup>99m</sup>Tc-GSH made from Lyophilized GSH kit.

The GSH kits prepared in a freeze-dried form (lyophilized kit) have a long shelf life, ranging from several months to years [10]. The expiration time of dried kits had to be determined to allow testing of the stability of the storage time. The expiration date of dried GSH kits can be determined by testing the radiochemical purity every month after labeled with <sup>99m</sup>Tc. After storage for 13 months, either at room temperature or 4°C, <sup>99m</sup>Tc-GSH still has radiochemical purity greater than 99% (Fig. 2). It can be stated that the dry GSH kit remained stable and still met the requirement of radiochemical purity being no less than 90% [5,9].

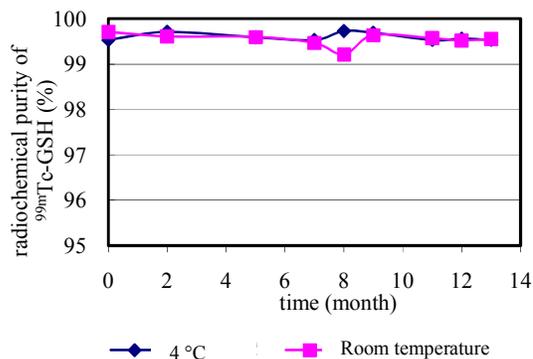


Fig. 2. Stability of Lyophilized GSH kit.

The results of the toxicity testing of <sup>99m</sup>Tc-GSH with five white mice are shown in Table 1. They indicate that no mice died or exhibited abnormalities within the observation period of 24 hours to seven days. It means that <sup>99m</sup>Tc-GSH is not toxic; thus, we continued to the next test.

Table 1. Abnormality conditions of test animals after <sup>99m</sup>Tc-GSH injection.

Mice	Day						
	1	2	3	4	5	6	7
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-

Description: (-) animals remain life and showed no symptoms of abnormality.

In the labeling of lyophilized kit with radionuclide <sup>99m</sup>Tc, to achieve the desired activity, sometimes a large volume of Na<sup>99m</sup>TcO<sub>4</sub> solution is required. Therefore, in this research, the influence of the volume and activity of Na<sup>99m</sup>TcO<sub>4</sub> solution to the radiochemical purity of <sup>99m</sup>Tc-GSH was investigated. The results are presented in Fig. 3, which shows that the use of 1-4 mL of Na<sup>99m</sup>TcO<sub>4</sub> solution resulted in radiochemical purity of <sup>99m</sup>Tc-GSH exceeding 99%. Radiochemical purity decreased to 97% when the volume of Na<sup>99m</sup>TcO<sub>4</sub> solution was increased 10 mL. Increasing the volume decreases reaction rate [11], and therefore not all of the existing <sup>99m</sup>TcO<sub>4</sub><sup>-</sup> in the solution was reduced to form <sup>99m</sup>TcO<sub>2</sub> or <sup>99m</sup>Tc-reduced and bound to GSH to form <sup>99m</sup>Tc-GSH. Nevertheless, the radiochemical purity of <sup>99m</sup>Tc-GSH still exceeded 90% which qualifies it as a good radiopharmaceutical [12].

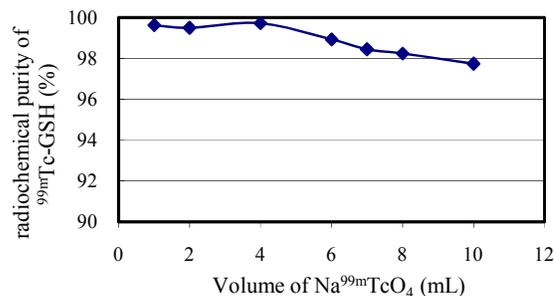


Fig. 3. Effect of volume Na<sup>99m</sup>TcO<sub>4</sub> used in the labeling of Lyophilized GSH kit to the radiochemical purity of <sup>99m</sup>Tc-GSH.

The investigation of the influence of activity was conducted using a fixed volume of 7 mL of Na<sup>99m</sup>TcO<sub>4</sub> with activities of 10, 20, 30, 40, 50 and 70 mCi. Table 2 shows that at the incubation time of 0 minutes, under activities of 10 and 20 mCi, the radiochemical purity greater was than 90%, while for 30, 40, 50 and 70 mCi, radiochemical purity was low, amounting to 54.58 ± 0.68%, 47.87 ± 0.77%, 33.83 ± 0.81% and 20.68 ± 0.78% respectively. When the incubation time was extended to 30 minutes, the radiochemical purity of <sup>99m</sup>Tc-GSH increased to more than 90%, except for the activity

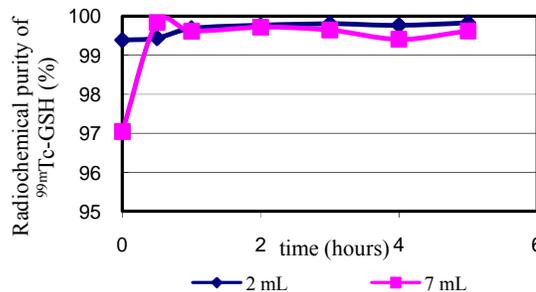
of 70 mCi, in which a longer incubation time, up to 3 hours, was required. The results also show that after the addition of  $\text{Na}^{99\text{m}}\text{TcO}_4$  with increased radioactivity, after a brief shaking (incubation time 0 minutes), the radiochemical impurities of  $^{99\text{m}}\text{TcO}_4^-$  increased. This means that there is still much  $^{99\text{m}}\text{Tc}$  (VII), which has not been reduced by the reductant Sn (II) to  $^{99\text{m}}\text{Tc}$ -reduced form which binds to glutathione (GSH) to form  $^{99\text{m}}\text{Tc}$ -GSH [5,6].

**Table 2.** The percentage of radiochemical impurities  $^{99\text{m}}\text{TcO}_4^-$  and  $^{99\text{m}}\text{TcO}_2$  contained in  $^{99\text{m}}\text{Tc}$ -GSH with the influence of  $\text{Na}^{99\text{m}}\text{TcO}_4$  radioactivity in labeling of lyophilized kit GSH.

Time (hour)	Activity (mCi)						
	10	20	30	40	50	70	
0	A	1.45	2.61	45.07	51.89	65.96	79.07
	B	0.09	0.36	0.39	0.24	0.21	0.25
1/2	A	0.10	0.08	0.32	0.64	7.26	55.41
	B	0.01	0.08	0.15	0.40	0.28	0.39
1	A	0.11	0.16	0.23	0.41	0.57	37.52
	B	0.10	0.22	0.18	0.30	0.52	0.32
2	A	0.06	0.17	0.34	0.15	3.08	15.00
	B	0.13	0.11	0.17	0.19	0.21	0.40
3	A	0.10	0.10	0.81	0.48	0.49	0.40
	B	0.08	0.25	0.93	0.40	0.30	0.30
4	A	0.04	0.19	0.25	0.30	1.93	0.28
	B	0.21	0.40	0.20	0.30	0.27	0.28
5	A	0.07	0.16	0.55	0.70	2.63	0.54
	B	0.05	0.20	0.42	0.25	0.25	0.27

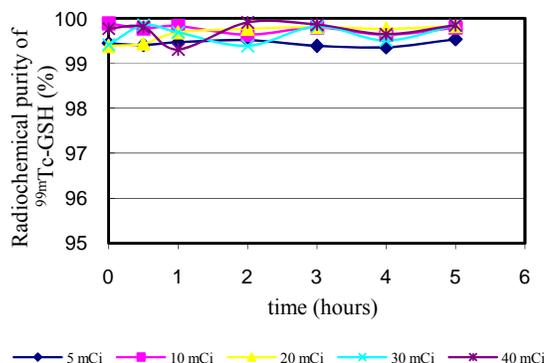
Description:  
 A :  $^{99\text{m}}\text{TcO}_4^-$  (%)  
 B :  $^{99\text{m}}\text{TcO}_2$  (%)

Figure 4 shows the comparison of pattern formation on  $^{99\text{m}}\text{Tc}$ -labeling dry kit GSH with  $\text{Na}^{99\text{m}}\text{TcO}_4$  in 2 and 7 mL of volume. The results showed that with the same incubation time, the radiochemical purity of  $^{99\text{m}}\text{Tc}$ -GSH with 7 mL volume was lower ( $97.04 \pm 1.50\%$ ) compared with  $^{99\text{m}}\text{Tc}$ -GSH with 2 mL volume ( $99.39 \pm 0.57\%$ ). When the incubation time was extended to 30 minutes, the radiochemical purities of both radiopharmaceuticals exceed 99% and are stable for the five hour incubation time. These results showed that the formation rate of  $^{99\text{m}}\text{Tc}$ -GSH was only weakly influenced by the volume.



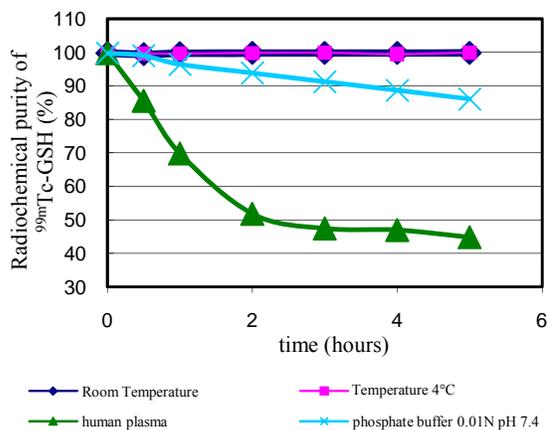
**Fig. 4.** Comparison of  $^{99\text{m}}\text{Tc}$ -GSH formation patterns in labeling of Lyophilized GSH kit using  $\text{Na}^{99\text{m}}\text{TcO}_4$  with different volume and radioactivity ( $\approx 20$  mCi).

The stability of the  $^{99\text{m}}\text{Tc}$ -GSH diagnostic kit could be obtained by observing the radiochemical purity for a specific duration. The results showed that when stored at room temperature for up to five hours, the radiopharmaceutical maintains a radiochemical purity above 99%, as shown in Fig. 5. From this analysis it can be stated that  $^{99\text{m}}\text{Tc}$ -GSH with activity in the 5 to 40 mCi range, when stored at room temperature, can still be used up to five hours after the addition of the  $^{99\text{m}}\text{Tc}$ .



**Fig. 5.** Stability of  $^{99\text{m}}\text{Tc}$ -GSH diagnostic kits made from the Lyophilized GSH kit in various concentrations of radioactivity.

The stability of  $^{99\text{m}}\text{Tc}$ -GSH in plasma is presented in Fig. 6. It shows that the radiochemical purity of  $^{99\text{m}}\text{Tc}$ -GSH in plasma decreased rapidly. After storage for 2 hours, radiochemical purity of  $^{99\text{m}}\text{Tc}$ -GSH was  $51.84 \pm 2.52\%$ , while after 3,4 and 5 hours, radiochemical purity did not significantly change further; they were  $47.43 \pm 0.08\%$ ,  $46.99 \pm 1.44\%$  and  $44.87 \pm 4.39\%$ , respectively. This result indicates that  $^{99\text{m}}\text{Tc}$ -GSH has a low stability in plasma. In contrast, when  $^{99\text{m}}\text{Tc}$ -GSH was stored in phosphate buffer for up to 3 hours, its radiochemical still exceeded 90% ( $91.24 \pm 4.39\%$ ).



**Fig. 6.** Stability of <sup>99m</sup>Tc-GSH made from lyophilized GSH kit in a variety of storage conditions.

## CONCLUSION

Dry kits of GSH have been produced by lyophilization. The kits are in the form of an odorless white solid which resembles cotton and are packaged in sterile vials under vacuum conditions. They are stable for 13 months of storage at room temperature and at 4°C.

<sup>99m</sup>Tc-GSH which was obtained from the lyophilized GSH kit has a radiochemical purity of  $99.54 \pm 0.01\%$ , is non-toxic and stable for up to 5 hours of storage at room temperature and at 4°C. The stability of <sup>99m</sup>Tc-GSH in blood plasma is less than those stored at room temperature and at 4°C; within two hours, the radiochemical purity decreases to  $51.84 \pm 2.52\%$ .

For volumes of  $\text{Na}^{99\text{m}}\text{TcO}_4$  exceeding 4 mL the radiochemical purity of <sup>99m</sup>Tc-GSH decreases. Further, the use of <sup>99m</sup>Tc with an activity greater than 20 mCi with a large volume (7 mL) results in the extension of the required incubation time to attain radiochemical purity  $\geq 90\%$ .

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