The Use of Low Ammonia Concentrations in the Radiochemical Purity Test of $[^{153}\text{Sm}]$Sm-EDTMP by Using the Thin Layer Chromatography Method

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ABSTRACT

Radiochemical purity testing of $[^{153}\text{Sm}]$Sm-EDTMP usually uses the Thin Layer Chromatography method. The mobile phase used is a mixture of 25% ammonia and water. However, the lowest ratio of 25% ammonia in the mobile phase is unknown. Therefore, research related to the use of the lowest concentration in the radiochemical purity test of $[^{153}\text{Sm}]$Sm-EDTMP is necessary. This research method includes labelling of EDTMP using Samarium-153, preparation of the mobile phase with variations in the concentration of 25% ammonia: water, radiochemical purity test and data analysis using t-test statistics. The results of this study are the concentration of 25% ammonia: water (1: 9) to (1: 200) still shows good separation with Rf of $[^{153}\text{Sm}]$SmCl$_3$ and $[^{153}\text{Sm}]$Sm-EDTMP at 0.0, 1.0 respectively, whereas with a thinner concentration of ammonia indicates less optimal separation with Rf $[^{153}\text{Sm}]$SmCl$_3$ at 0.35 to 1.0. Comparison of concentrated ammonia concentrations of 1: 9 and dilute 1: 200 was performed using a statistical t-test. The results of the data analysis showed that the two methods were not significantly different, indicated by the t-value of 0.82 less than 2.78. The conclusion of this study is that the lowest concentration of 25% ammonia and water in the radiochemical purity test of $[^{153}\text{Sm}]$Sm-EDTMP is 1: 200.

Keywords: Radiochemical purity, $[^{153}\text{Sm}]$Sm-EDTMP, Thin Layer Chromatography, Ammonia Concentration, Mobile phase

INTRODUCTION

Samarium-153 Ethylenediamine Tetramethylene Phosphonate (EDTMP) or $[^{153}\text{Sm}]$Sm-EDTMP is a radiopharmaceutical in the form of a sterile solution that has a half-life of 1.9 days with beta energy of 634 KeV, 703 KeV, 807 KeV [1,2,3]. Based on the energy possessed $[^{153}\text{Sm}]$Sm-EDTMP can be indicated to overcome the pain of palliation in cancer patients who have metastasized to the bone [4,5,6]. $[^{153}\text{Sm}]$Sm-EDTMP will be distributed into the bone, especially in tissues with high osteoblastic activity. In metastatic bone tissue, the concentration of $[^{153}\text{Sm}]$Sm-EDTMP is five times higher than normal tissue, so it is effective in the process of palliative pain therapy in bone cancer [7].

In determining the accuracy of $[^{153}\text{Sm}]$Sm-EDTMP for targeted organs, it is necessary regarding the quality control of this radiopharmaceutical [8,9]. Quality control parameters in

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radiopharmaceutical preparations are generally almost the same as other sterile preparations such as visual observation, pH measurement, radionuclide purity, radiochemical purity, radioactive concentration and sterility testing using endotoxin bacterial testing [10]. The most important QC parameter to see how radiopharmaceuticals reach the target is radiochemical purity. In general, the higher radiochemistry of a radiopharmaceutical, resulting in the better imaging [11,12]. The permissible radiochemical purity of $^{153}$SmEDTMP is greater than 95%. In a previous study by Kadarisman et al. radiochemical testing of $^{153}$SmEDTMP using a mixture mobile phase, which is 32% ammonia solution: methanol: water (1: 10: 20) [13]. In pharmacopoeia issued by WHO, the $^{153}$SmEDTMP radiochemical purity testing uses Thin Layer Chromatography (TLC) method with a mixture of mobile phase solvent of 0.2 volumes of 26% ammonia and 40 volumes of water with the stationary phase of Whatman No. 1 paper chromatography. The use of this TLC method provides a reference to the Retention Factor (Rf) value of 0.0 in $^{153}$SmCl$_3$ impurities, while the $^{153}$SmEDTMP Rf value is 0.6 [14]. In general, the $^{153}$SmEDTMP radiochemical purity test can use the ammonia concentration in its mobile phase. However, the use of low concentrations with a combination of 25% ammonia and water has never been tested, so this test can show the minimum concentration that can be used for $^{153}$SmEDTMP radiochemical testing.

In addition, ammonia solution has volatile characteristics to all surfaces so that it can irritate the eyes and respiratory tract. This is quite disturbing to the analyst when conducting radiochemical purity testing [15]. Therefore, a more efficient $^{153}$SmEDTMP radiochemical purity testing method is required when conducting analyses. The purpose of this study was to determine the ratio of the lowest ammonia concentration in the radiochemical purity testing of $^{153}$SmEDTMP as the mobile phase with ammonia: water variations.

In this study, the radiochemical purity test of $^{153}$SmEDTMP was carried out using the TLC method and a mixture of ammonia: water, 1: 9, 1: 100, 1: 150, 1: 200, 1: 1000, 1: 2000, 1: 5000, 1: 30000, and water 100 % as the mobile phase.

**EXPERIMENT**

**Chemicals and instrumentation**

The materials used in this study were labelled compound $^{153}$SmEDTMP produced by the Center for Radioisotope and Radiopharmaceutical Technology – National Nuclear Energy Agency (PTRR-BATAN), water for injection (IPHA Laboratories), 25% ammonia solution (Merck), plastic film, Whatman No. 1 paper with a size of 1 x 12 cm (Sigma-Aldrich). The tools used in this research were Chromatographic cylinder vessels, micropipette, and tips (Eppendorf), radiochromatography scanner (TLC Scanner 204 Comecer).

**Labelling EDTMP using Samarium-153**

The labelling of EDTMP using Samarium-153 began with the irradiation of natural Sm$_2$O$_3$ as raw material in the G.A Siwabessy Multipurpose Reactor (PRSG-GAS BATAN). The irradiated target material was dissolved in 1N HCl and produced $^{153}$SmCl$_3$. Labelling process was carried out by adding EDTMP dissolved in 1N NaOH to $^{153}$SmCl$_3$ until the pH reached 7.0 - 8.5 and stirred for ± 60 minutes at room temperature.
Mobile Phase Preparation

The mobile phase was 25% ammonia solution and water with a ratio of 1:9; 1:100; 1:150; 1:200; 1:1,000; 1:2,000; 1:5,000; 1:30,000 and 100% water put into each cylinder chromatographic vessel.

Radiochemical Purity Test

An aliquot amount of $^{153}$Sm-EDTMP was pipetted and spotted on the baseline (2 cm from the lower end) of Whatman No.1 paper. The paper was inserted into the chromatographic cylinder vessel until the paper touches the surface of the mobile phase. The mobile phase would migrate until it reached 1 cm from the top of the Whatman No.1 paper strip. Removed and dried immediately at room temperature, and put in plastic. The plastic containing Whatman paper was measured by radiochemical purity using a TLC Scanner. Radiochemical purity experiments and measurements were repeated with the same stages and process conditions.

Data Analysis

The result of optimizing the $^{153}$Sm-EDTMP radiochemical purity test was the discovery of a method that minimizes the use of ammonia solution. Therefore, testing with concentrated and thinner ammonia was carried out. The experiment was carried out three times with five replications. Furthermore, the data were be tested statistically using the t-test.

RESULT AND DISCUSSION

In this study, a radiochemical purity testing $^{153}$Sm-EDTMP with Whatman No.1 paper as stationary phase and variation of 25% ammonia: water and also 100% water as mobile phase. The concentration of ammonia in the mobile phase in this test greatly affected the resulting radiochromatogram of $^{153}$Sm-EDTMP as shown in figure 1.

![Radiochromatogram results from the separation of $^{153}$Sm-EDTMP and $^{153}$SmCl$_3$ with the stationary phase of Whatman No. 1 and mobile phase of 25% ammonia: water (1:200)](image-url)

**Figure 1.** Radiochromatogram results from the separation of $^{153}$Sm-EDTMP and $^{153}$SmCl$_3$ with the stationary phase of Whatman No. 1 and mobile phase of 25% ammonia: water (1:200)
Figure 1 shows the results of an $^{153}\text{Sm}$Sm-EDTMP radiochromatogram with a mixture of 25 % ammonia: water (1: 200). The rf value of the $^{153}\text{Sm}$Sm-EDTMP product is at 1.0 because edtmp is an organic compound that has a polarity level that can be eluted by a mixture of 25 % ammonia and water reagents (1: 200). Meanwhile, $^{153}\text{Sm}$SmCl$_3$ impurities have lower polarity so that these compounds do not migrate on Whatman paper No. 1. This is caused by Ammonia is a polar compound that has one electron pair at the center atom. The pair of electrons again bends the bonds and stay on top. Although ammonia and water are both polar in nature, in their development these solutions are known that not only have different critical values but also different polarities [15]. The ratio of 25 % ammonia and water concentrations (1: 200) can separate $^{153}\text{Sm}$Sm-EDTMP with their impurities $^{153}\text{Sm}$SmCl$_3$. The function of ammonia here is to increase separation of $^{153}\text{Sm}$Sm-EDTMP compound efficiency [17].

Separation systems using TLC typically use a single or mixed mobile phase whose polarity is in accordance with the compound to be separated. Polar compounds will be withdrawn with a solvent that has polar properties as well [16]. Based on Figure 2, $^{153}\text{Sm}$Sm-EDTMP is polar because it has O-H chemical bonds outside its chemical structure with a difference in electronegativities of 1.24 eV. However, $^{153}\text{Sm}$SmCl$_3$ is more polar than $^{153}\text{Sm}$Sm-EDTMP because there is a Sm-Cl covalent bond with a difference in electronegativities of 1.99 eV. Based on this polarity difference, the $^{153}\text{Sm}$Sm-EDTMP and $^{153}\text{Sm}$SmCl$_3$ compounds can be separated using a ratio of 25% ammonia and water (1: 200). The more dilute the ratio of ammonia ratio of 25% and water, the higher the Rf value of SmCl$_3$, while the $^{153}\text{Sm}$Sm-EDTMP compound remains in the front solvent Rf 1.0.

A comprehensive Rf value of $^{153}\text{Sm}$Sm-EDTMP and $^{153}\text{Sm}$SmCl$_3$ impurities are shown in Figure 3. It is shown $^{153}\text{Sm}$SmCl$_3$ has a varying Rf value depending on the concentration of ammonia as a mobile phase. The separation in the test is said to be good if the Rf of $^{153}\text{Sm}$Sm-EDTMP is at 1.0 and the impurity $^{153}\text{Sm}$SmCl$_3$ is at Rf 0.0. The Rf value of $^{153}\text{Sm}$Sm-EDTMP is 1.0 for all mixed phases of ammonia and water. The appearance is different in the Rf value of $^{153}\text{Sm}$SmCl$_3$ according to the concentration of ammonia as a mobile phase. The mixture of solvents having ammonia with a higher concentration of 1: 9 to 1: 200 showed Rf value of 0.0 while the mobile phase with a lower ammonia concentration was shown at concentrations of 1: 1,000, 1: 2000, and 1: 5,000 the Rf value moved from 0.35, 0.55 and 0.60, respectively. The Rf value of $^{153}\text{Sm}$SmCl$_3$ at the lowest ammonia concentration of 1: 30,000 shows at 1.0 equal to the 100% water phase. This occurs because of the change at mixture of ammonia and water polarity, the higher ammonia concentration in mobile phase of ammonia and water, a good separation from $^{153}\text{Sm}$Sm-EDTMP radiochemical purity shown by the Rf value of 0.0.
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Based on the above test results, it can be compared with the results of radiochemical purity $^{153}$Sm-Sm-EDTMP using concentrated ammonia concentration of 1: 9 with a dilute concentration of 1: 200. A comparison of the two methods was done by testing $^{153}$Sm-Sm-EDTMP for three measurements and analyzed using a t-statistic test. The results of data analysis are shown in table 1.

Table 1. Evaluation of radiochemical purity data of $^{153}$Sm-Sm-EDTMP with a comparison of ammonia: water as mobile phase (1: 9) and (1: 200)

| Trial | Percentage of Radiochemical Purity of $^{153}$Sm-Sm-EDTMP |
|-------|--------------------------------------------------------|
|       | 25% ammonia: water (1:9) | 25% ammonia: water (1:200) |
| 1     | 99.95 ± 0.04 | 99.97 ± 0.03 |
| 2     | 99.84 ± 0.30 | 99.63 ± 0.56 |
| 3     | 99.93 ± 0.05 | 99.86 ± 0.23 |
| Average | 99.91 | 99.82 |
| SD    | 0.06 | 0.18 |
| p-value | 0.46 | |
| t-stat value | 0.82 | |

Data analysis using a statistical t-test is needed to see whether the two methods show significantly different results or not. In table 1 compared the radiochemical purity of $^{153}$Sm-Sm-EDTMP using a concentrated mobile phase of 25% ammonia: water (1: 9) and thinner (1: 200). The mean radiochemical purity of $^{153}$Sm-Sm-EDTMP in 25% ammonia: water (1: 9) was 99.91 ± 0.06 and at (1: 200) it was 99.82 ± 0.18. This value still shows good repeatability with a small standard deviation of less than 1.

Hypothesis of 0: 25% ammonia: water (1: 9) method is not significantly different from the 25% ammonia: water (1: 200) method if the p-value is greater than 0.05 and the t-stat value is less than 2.78. Hypothesis of 1: 25% ammonia: water (1: 9) method is significantly different
from the 25% ammonia: water (1: 200) method if the p-value is less than 0.05 and the t-stat value is greater than 2.78.

In analyzing data using the statistical t-test, it can be seen that the p-value of 0.46 is more than 0.05 and the t-stat value is less than 2.78 which indicates that the radiochemical purity testing method $^{153}$Sm-EDTMP uses a mobile phase of 25% ammonia: water (1: 9 ) and (1: 200) show that are not significantly different.

The radiochemical purity testing method of $^{153}$Sm-EDTMP with a mobile phase of 25% ammonia: water (1: 200) is also more efficient with an rf value of 1.0, radiochemical purity of 99.82 ± 0.18 compared to previous research methods using the mobile phase of ammonia: methanol: water (1:10:20) with the product rf value of 0.7 - 0.8, radiochemical purity of 96.59 ± 1.24 % [13]. The method using solvent mixture 25 % ammonia: water (1: 200) has a low deviation indicates the consistency of the measurement results, the perfect separation between the products at rf 1.0 and impurity 0.0, and the use of ammonia compounds with low concentrations can be used.

CONCLUSION

Based on the data, it can be concluded that the lowest concentration of ammonia that can be used in the radiochemical purity test of $^{153}$Sm-EDTMP is 25% ammonia: water (1: 200). This mobile phase can separate $^{153}$Sm-EDTMP and $^{153}$SmCl₃ well at Rf 1.0 and 0.0, respectively.

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