

Determination of Radiochemical Purity of ^{99m}Tc -DTPA Using One-System Method of Paper Chromatography

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ABSTRACT

The more efficient and effective quality control techniques for ^{99m}Tc -DTPA are needed because ^{99m}Tc has a short half-life of around 6.0 hours. We have succeeded in developing a one-system of Thin Layer Chromatography (TLC) for radiochemical purity testing system that is faster and more practical. Two-system method of TLC for radiochemical purity testing uses mobile phase of methyl ethyl ketone indicated as system A and 0.9% sodium chloride solution indicated as system B. One-system method uses the mobile phase of a mixture solution of acetone and 0.9% sodium chloride. In this study, the determination of radiochemical purity of the one-system of TLC has been successfully developed using the Whatman-1 paper stationary phase and the mixture of mobile phase between acetone and 0.9% sodium chloride solution. The mobile phase of acetone: 0.9% sodium chloride with a ratio of 9:1 shows the most optimum results. This phase can separate ^{99m}Tc -DTPA ($R_f = 0.4$ - 0.6) from $^{99m}\text{TcO}_4^-$ ($R_f = 0.9$ - 1.0) and $^{99m}\text{TcO}_2$ ($R_f = 0.0$ - 0.1) as radiochemical impurities. This result shows that the one-system of TLC method can be used for radiochemical purity testing of ^{99m}Tc -DTPA radiopharmaceutical kits. This method can completely separate the product compound (^{99m}Tc -DTPA) from its impurities ($^{99m}\text{TcO}_2$ and $^{99m}\text{TcO}_4^-$).

Key word: Radiochemical purity, ^{99m}Tc -DTPA, ^{99m}Tc , one-system of TLC, two-system of TLC.

INTRODUCTION

Every year, the number of cases of noninfectious diseases continue to increase in Indonesia. One of them is kidney disease which ranks third in terms of the number of cases and the cost of treatment after stroke and heart disease [1]. The kidneys are important organs in the body, especially for removing metabolic wastes and toxins in the blood through urine. If the kidneys do not work properly, they can have serious consequences for the body's condition [2-3]. Chronic Renal Failure (CRF) is a clinical syndrome caused by a chronic decline in kidney function, progressively and irreversibly [4-6].

The Glomerular or tubulointerstitial disease is a disorder of the kidneys that can be cured, but if it occurs repeatedly and chronically, it will reduce kidney function. The glomerular or tubulointerstitial disease suffered by the patients can increase the risk of Chronic Kidney Disease (CKD) [7]. In developing countries, CKD is generally caused by infection or exposure to drugs or toxic substances [8]. End-stage of the CKD is caused by glomerulonephritis, obstruction kidney disease & infection, diabetic kidney disease, hypertension, and polycystic

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kidney [7], [9]. In addition to diseases that occur directly in the kidney, hypertension and diabetes mellitus are also risk factors for CKD [7], [10]. Glomerular filtration rate can be measured using ^{99m}Tc -DTPA radiopharmaceuticals [11-14].

Accurate and early diagnosis is very important for the treatment step, otherwise, a wrong diagnosis always has negative consequences for the quality of life of the patients [3]. Nowadays, nuclear medicine has an important role in the diagnosis and treatment of many diseases [15-17]. The ^{99m}Tc -based radiopharmaceuticals are ideal radiopharmaceuticals for diagnosis because ^{99m}Tc radioisotope has a short half-life (6 hours). In addition, the ^{99m}Tc radioisotope is a pure gamma-ray transmitter (140 keV) which is ideal for producing good imaging using SPECT (Single Photon Emission Computed Tomography) devices [15-16].

Quality control is absolutely necessary before the radiopharmaceuticals are used to the patients. One of the important parameters in quality control is the Radiochemical Purity (RCP) test because it will affect radiopharmaceuticals biodistribution. The purer the radiochemical purity, the easier the radiopharmaceuticals are distributed towards the target organ [17]. Generally, the radiochemical purity test is carried out by using a chromatographic method [18]. Based on the European Pharmacopoeia, Instant Thin Layer Chromatography Medium-Silica Gel (ITLC-SG) is used to perform the ^{99m}Tc -DTPA radiochemical purity test. This method is very fast and easy, but since 2008 PALL Corporation did not produce ITLC anymore and then scientists look for an alternative method [19-20]. In 2013, Maria C.B. *et. al* has conducted validation of alternative methods for testing of ^{99m}Tc -DTPA radiochemical purity [21].

In general, the method used in determining of the radiochemical purity of the radiopharmaceutical products is the two-system chromatography method, where the first system is to determine the $^{99m}\text{TcO}_2$ impurity, and the second system is to determine the amount of $^{99m}\text{TcO}_4^-$ impurity. Furthermore, the radiochemical purity of the radiopharmaceutical complex compound was calculated. The purpose of this study is to prove whether one-system method can be used in the radiochemical separation method ^{99m}Tc -DTPA, and impurities ($^{99m}\text{TcO}_2$ and $^{99m}\text{TcO}_4^-$).

EXPERIMENT

Materials

The materials used were radiopharmaceutical kits DTPA produced by National Nuclear Energy Agency (BATAN) and PT Kimia Farma, Indonesia, acetone for analysis 99.8% and methyl ethyl ketone 99.5% (Merck), 0.9% sodium chloride (Otsuka), sterile water (IPHA), and Technetium-99m (BATAN).

Equipment

The equipment used was vortex (Thermo), chromatography vessel, Gamma Ionization Chamber (Biodex Atomlab 300), Caprac-t Gamma Counter (Nucleus).

Labelling of Radiopharmaceuticals

Labelling of ^{99m}Tc -DTPA was carried out by mixing of DTPA radiopharmaceutical kit with 37-740 MBq (2-3 ml) ^{99m}Tc -pertechnetate solution obtained from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator (BATAN), shaken until dissolved, then incubated at room temperature for 10 minutes.

Study of Radiochemical Purity Test Methods

Radiochemical purity test was carried out by two TLC methods, namely two-system method of paper chromatography which was a well-established method and another system of paper chromatography which was an innovation method.

Radiochromatography of the two-system method

System A. Determining of ^{99m}Tc Pertechnetate radiochemical impurities

An amount of 1 μL of ^{99m}Tc -DTPA was spotted on Whatman-1 paper at a distance of 2.5 cm (1x13 cm size) and left to dry. Then, the radiochromatography paper was eluted using a methyl ethyl ketone solution and was removed when the eluent had moved up 10 cm from the initial spotted point. Then the radioactivity of the chromatogram was counted by using gamma counter. In this radiochromatography A, free ^{99m}Tc -Pertechnetate ($^{99m}\text{TcO}_4^-$) would be carried by eluent ($R_f = 0.9 - 1.0$), while both of ^{99m}Tc -DTPA and $^{99m}\text{TcO}_2$ remained at initial spotted point ($R_f = 0 - 0.1$) so that the free ^{99m}Tc -Pertechnetate as a radiochemical impurity could be calculated.

System B. Determining of $^{99m}\text{TcO}_2$ Radiochemical Impurities

An amount of 1 μL of ^{99m}Tc -DTPA was spotted on Whatman-1 paper at a distance of 2.5 cm (1x13 cm size) and left to dry. Then, the radiochromatography paper was eluted using a 0.9% sodium chloride solution and was removed when the eluent had moved up 10 cm from the initial spotted point. Then the radioactivity of the chromatogram was counted by using gamma counter. In this radiochromatography B, ^{99m}Tc -pertechnetate free ($^{99m}\text{TcO}_4^-$) and ^{99m}Tc -DTPA would be carried by eluent ($R_f = 0.9 - 1.0$), while $^{99m}\text{TcO}_2$ would remain at the initial bottling ($R_f = 0-0.1$) so that $^{99m}\text{TcO}_2$ as radiochemical impurities could be calculated. Furthermore, ^{99m}Tc -DTPA radiochemical purity was calculated using the following equation 1 [1].

$$\%^{99m}\text{TcDTPA} = 100 - \%^{99m}\text{TcO}_4^- - \%^{99m}\text{TcO}_2 \quad (1)$$

Radiochromatography one-system method

One-system method of paper chromatography was a further development of the two-system method. In the two-system method, separated eluents were used in the chromatographic vessel, whereas in one-system method eluents were combined in one chromatographic vessel in order to separate 3 components at once, namely $^{99m}\text{TcO}_2$, $^{99m}\text{TcO}_4^-$, and ^{99m}Tc -DTPA. The amount 1 μL of ^{99m}Tc -DTPA was spotted on Whatman-1 paper at a distance of 2.5 cm (1x13 cm size) and left to dry. Afterwards, the radiochromatography paper was eluted using a mixture of Acetone and 0.9% sodium chloride solution, then lifted when the eluent had moved 10 cm from the initial spotted point. It was then calculated using the gamma counter. In this study, the eluent acetone and 0.9% sodium chloride were varied in a ratio of 0: 10, 1: 9, 3: 7, 5: 5, 5.5: 4.5, 7: 3, 9: 1 and 10:0.

RESULT AND DISCUSSION

In this study, the determination of ^{99m}Tc -DTPA radiochemical purity using the TLC method innovation with the modified mobile phase has been done. The method modification is to combine two types of mobile phases, namely Acetone and 0.9% sodium chloride solution with certain mixture compositions so that they can separate ^{99m}Tc -DTPA, $^{99m}\text{TcO}_2$ and $^{99m}\text{TcO}_4^-$.

The two-system method of radiochemical purity test of ^{99m}Tc -DTPA radiopharmaceuticals kits are shown in Figures 1 and 2.

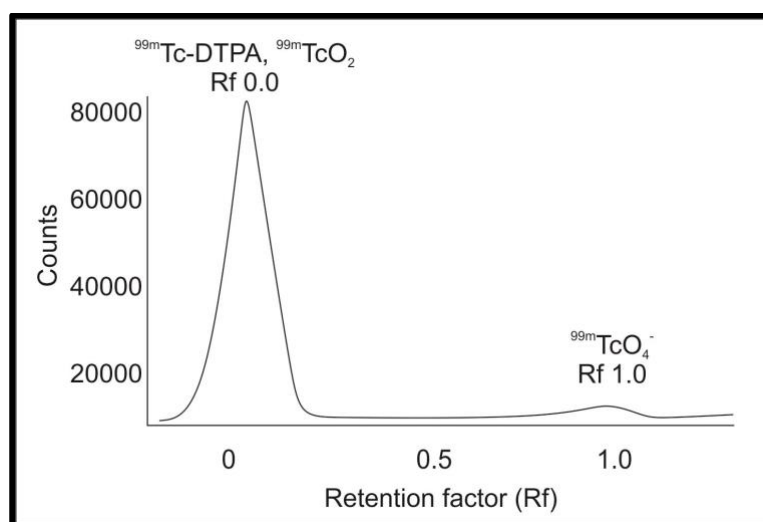


Figure 1. Radiochromatogram using Whatman-1 paper as stationary phase and methyl ethyl ketone as mobile phase (TLC A)

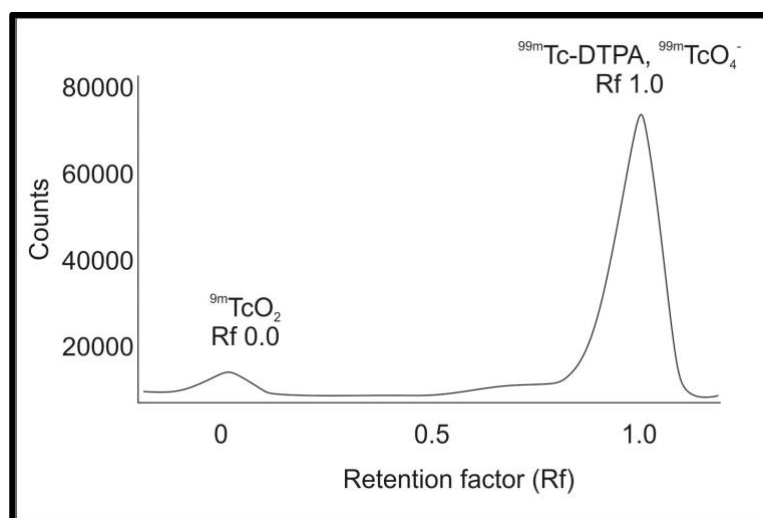


Figure 2. Radiochromatogram using Whatman-1 paper as stationary phase and 0.9% sodium chloride as mobile phase (TLC B)

Figure 1 shows that by using Whatman-1 paper as stationary phase and methyl ethyl ketone as mobile phase (also known as TLC A in this paper), $^{99m}\text{TcO}_4^-$ was identified at $R_f = 0.7-0.9$, $^{99m}\text{TcO}_2 = 0.0-0.1$, and $^{99m}\text{Tc-DTPA} = 0.0-0.1$. Figure 2 shows the results of chromatography using Whatman-1 as stationary phase and 0.9% sodium chloride as mobile phase (also known as TLC B in this paper), where $^{99m}\text{TcO}_4^-$ is identified at $R_f = 0.8-1.0$, $^{99m}\text{TcO}_2 = 0.0-0.1$, and $^{99m}\text{Tc-DTPA} = 0.8-1.0$. These data indicate that both of the paper chromatography systems cannot separate the components of the ^{99m}Tc -DTPA radiochemical complex compound from

radiochemical impurities ($^{99m}\text{TcO}_2$ and $^{99m}\text{TcO}_4^-$). Paper chromatography A can determine only the percentage of $^{99m}\text{TcO}_4^-$ radiochemical impurity, while $^{99m}\text{Tc-DTPA}$ mixes with $^{99m}\text{TcO}_2$ at $R_f = 0.0-0.1$. Whereas from TLC B, the percentage of $^{99m}\text{TcO}_2$ as radiochemical impurities can be known, while $^{99m}\text{Tc-DTPA}$ mixed with $^{99m}\text{TcO}_4^-$ at $R_f = 0.8-1.0$.

The calculation of $^{99m}\text{Tc-DTPA}$ radiochemical purity was obtained by 100% minus the number of per cent of radiochemical impurities of $^{99m}\text{TcO}_4^-$ and $^{99m}\text{TcO}_2$. The calculation of the radiochemical purity of $^{99m}\text{Tc-DTPA}$ is obtained by subtracting 100% by the number of per cent of the radiochemical impurities of $^{99m}\text{TcO}_4^-$ and $^{99m}\text{TcO}_2$.

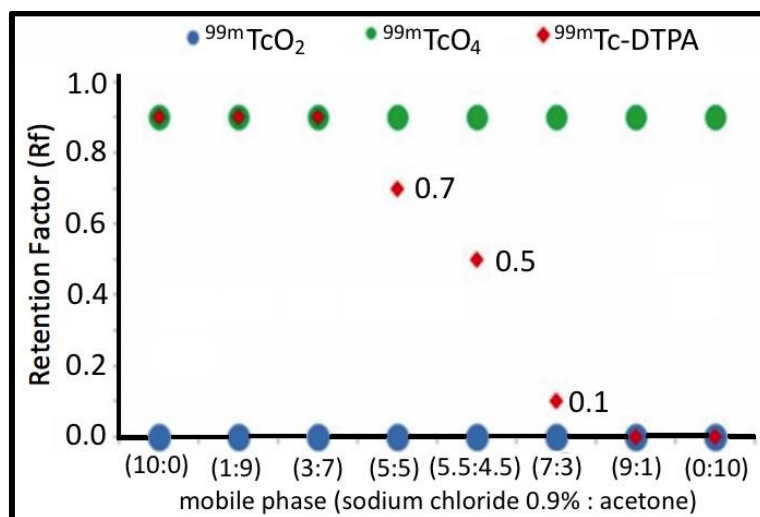


Figure 3. Optimization of the mobile phase composition between acetone and 0.9% sodium chloride

Recently, the two-system method of radiochemical purity testing is a standard method commonly used since long time ago [22] in Center for Radioisotope and Radiopharmaceutical Technology (PTRR) - BATAN or in various other laboratories abroad [23]. However, this method needs to be developed to make it easier and more efficient. In this study, we have successfully developed the paper chromatography one-system method for the $^{99m}\text{Tc-DTPA}$ radiochemical purity test. This method mixes two mobile phases with certain compositions in a chromatographic chamber. This method is used to separate $^{99m}\text{Tc-DTPA}$ radiochemistry from radiochemical impurities. Figure 3 shows the results of the optimization of the mobile phase composition between acetone and 0.9% sodium chloride.

In this study, varying the composition of the mobile phase was carried out in order to separate $^{99m}\text{Tc-DTPA}$, $^{99m}\text{TcO}_4^-$ and $^{99m}\text{TcO}_2$. Figure 3 shows the variation of the mobile phase acetone and 0.9% sodium chloride with ratio of 0:10, 1:9, 3:7, 5:5, 5.5:4.5, 7:3, 9:1 and 10:0. For eluent mixture with ratio of 1:9 and 3:7 shows that the chromatogram $^{99m}\text{Tc-DTPA}$ coincides with $^{99m}\text{TcO}_4^-$ at R_f 0.9. The optimal composition is acetone and 0.9% sodium chloride with a ratio of 5.5: 4.5. All three components ($^{99m}\text{Tc-DTPA}$, $^{99m}\text{TcO}_2$, and $^{99m}\text{TcO}_4^-$) can be completely separated by this eluent comparison.

Figure 4 shows that elution using acetone: 0.9% sodium chloride (5.5:4.5) could separate $^{99m}\text{Tc-DTPA}$, $^{99m}\text{TcO}_2$ and $^{99m}\text{TcO}_4^-$ very well. The $^{99m}\text{Tc-DTPA}$ chromatogram has R_f of 0.5 and it is completely separated from the impurities of $^{99m}\text{TcO}_4^-$ ($R_f = 0.9$) and $^{99m}\text{TcO}_2$ ($R_f = 0$). This result shows that the paper chromatography of one-system method can be used to

determine the radiochemical purity of ^{99m}Tc -DTPA radiopharmaceutical kits because it can completely separate among the product compound (^{99m}Tc -DTPA) and the impurities ($^{99m}\text{TcO}_2$ and $^{99m}\text{TcO}_4^-$).

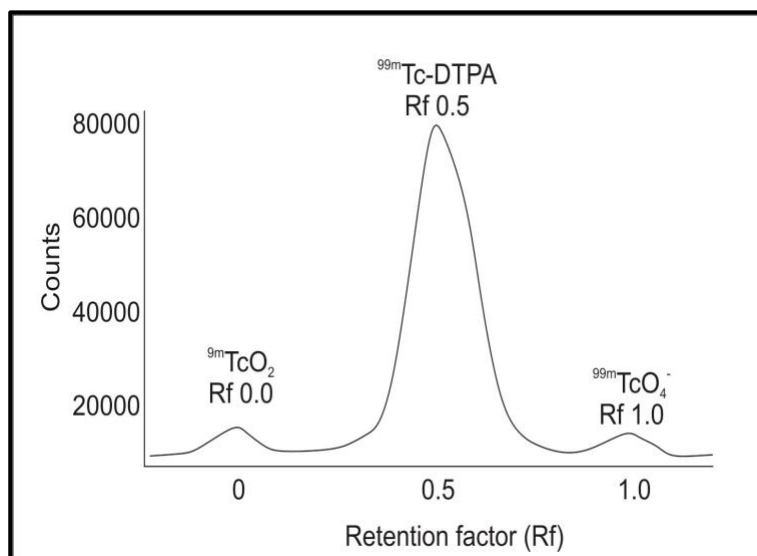


Figure 4. Radiochromatogram using Whatman-1 paper as stationary phase and acetone: 0.9% sodium chloride (5.5: 4.5) as mobile phase.

The summary of the comparison between the determination of radiochemical purity of one and two-system methods of paper chromatography is shown in Table 1. It shows that one-system of TLC give a better method than paper chromatography testing which generally uses two-system of TLC method. One-system of TLC method is easier and simpler because it only needs to be eluted once which eventually can directly separate between the product and its impurities, whereas the two-system of TLC cannot separate them. In addition, fewer types of equipment and measurement time are required.

Table 1. Determination of radiochemical purity of one and two system radiochromatography methods

Radiochemical	Retention Factor		
	Two-system of TLC		one System of TLC
	methyl ethyl ketone	0.9% sodium chloride	acetone:0.9% sodium chloride (11:9)
$^{99m}\text{TcO}_4^-$	0.7-0.9	0.8-1.0	0.8-1.0
$^{99m}\text{TcO}_2$	0.0-0.1	0.0-0.1	0.0-0.1
$^{99m}\text{Tc-DTPA}$	0.0-0.1	0.8-1.0	0.4-0.6

CONCLUSION

This study has successfully developed the determination of radiochemical purity of the one-system which is easier, more simple and effective. This method uses the Whatman-1 paper as the stationary phase and mixture between acetone and 0.9% sodium chloride as the mobile

phase. The optimal ratio of acetone: 0.9% sodium chloride is 5.5: 4.5. With this comparison, ^{99m}Tc -DTPA (Rf = 0.4-0.6) can be separated well from $^{99m}\text{TcO}_4^-$ (Rf = 0.9-1.0), and $^{99m}\text{TcO}_2$ (Rf 0.0-0.1) as radiochemical impurities.

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CONFLICT OF INTEREST

Authors declare no competing interests.

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