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Comparison of Measurement Method for Radiochemical Purity Determination of ^{99m}Tc-Methoxy Isobutyl Isonitrile

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Abstract

Globally, the death prevalence from heart disease accounts for 9.4 million deaths a year and will have predicted by 2030 increased to 23.6 million people suffering from heart disease. To solving this problem not only focus on treatment the disease but we should also think how to preventing it. Cardiac diagnosis has been recognized since 1989 using technetium-99m-methoxy isobutyl isonitrile (^{99m}Tc-MIBI) radiopharmaceutical. Since 2012, Indonesia has been producing about 500 vials MIBI kits (precursor of ^{99m}Tc-MIBI) per year. Since radiolabeling of MIBI kit with ^{99m}Tc is performed in hospital, therefore the quality control (QC) of the resulted product (^{99m}Tc-MIBI) should be performed at the hospital as well. The extraction method is a simple, fast, and friendly used method for determining radiochemical purity (RCP) of ^{99m}Tc-MIBI. However, this method still has some weaknesses as the sample volume and type of measurement tools greatly influence the results. Therefore, the aim of this study is to compare the results of RCP measurements of ^{99m}Tc-MIBI used by extraction method which is then measured with a dose calibrator (DC) and a gamma counter (GC), as well as thin layer chromatography (TLC) method. The research involved radiolabeling MIBI kit with ^{99m}Tc, testing RCP with TLC standard, DC and GC methods, and then comparing RCP ^{99m}Tc-MIBI results in those three methods with statistical calculation. The chromatogram resulted from TLC method showed ^{99m}Tc-MIBI (Rf 1.0) nicely separate from its impurities $^{99m}TcO_2$ (Rf 0.0) and $^{99m}TcO_4^-$ free (Rf 0.4 – 0.6). Minimum sample volume that can be well measured on DC and GC tools on extraction method is 250µL. The percentages of RCP of ^{99m}Tc-MIBI measured using TLC, DC, and GC methodsare 97.84 ± 0.49%, 97.45 ± 0.53%, and 97.95 ± 0.64% respectively. Comparison of extraction method with two different measurement tools, dose calibrator and gamma counter, was found not significantly different based on statistical calculation with t-value of 1.98. In addition, comparison between thin layer chromatography and gamma counter was found not also significantly different either with t-value of 0.46. Based on the above-mentioned results it can be concluded that the extraction method using two type tools, dose calibrator and gamma counter, do not differ significantly with the standard method TLC.

INTRODUCTION

Heart disease is one of the most deadly diseases in the world. There are approximately 9.4 million death a year and as many as 45% of deaths are caused by coronary heart disease (CHD). According to the Indonesia basic health research in 2013 that prevalence of CHD is at seventh highest position of non-communicable diseases [1]. It is estimated that by 2030 will increase to 23.6 million people of the world suffering from heart disease [2]. Classification of heart diseases is coronary heart disease, ischemic, stroke, heart attack, and atherosclerosis [3,4,5].

Health care services should not only think about modern heart disease therapy, but the right diagnosis is also needed to be considered for providingother therapeutic measures. In 1960s, nuclear medicine started to

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developrapidly in Indonesia [6]. Radiopharmaceuticals labeled by technetium-^{99m} (^{99m}Tc) occupy the top position in terms of application for imaging heart, brain, thyroid, lung, gallbladder, kidney, and some types of tumour [7]. The reason is ^{99m}Tc has a fast half life of about 6.0 hours with gamma energy around 140 KeV [8]. Therefore, radiopharmaceutical labeled ^{99m}Tc has a low risk for patients as it has low radiation doses and the time of patient isolation also becomes shorter [8].

Since 1989 methoxy isobutyl isonitrile labeled ^{99m}Tc (^{99m}Tc-MIBI) has been applied as a diagnostic radiopharmaceutical for cardiac perfusion [9]. Application of ^{99m}Tc-MIBI also reported as imaging of tumors and differentiation agents by Widyastuti *et,al*. [10]. In Indonesia since 1997 MIBI kits have been developed by National Nuclear Energy Agency (BATAN) and in 2012 have been successfully granted a production license by National Agency of Drug and Food Control (BPOM).In 2017 MIBI kits production reached of 500 vials of kits [11,12].

Quality control (QC) is the most important part in current good manufacturing practice (c-GMP) of pharmaceutical product because this factor will determine whether the pharmaceutical product conform to the required specification. One of the QC parameter is the percentage of radiochemical purity (RCP) which has not to be less than 90%, in order to give a good imaging [13,14]. If the RCP less than that of requirement, the imaging results will be not good due to the accumulation of impurities in the non-target organs. The impurity of ^{99m}Tc labeled radiopharmaceutical is free ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) and ^{99m}Tc in hydrolysed form ($^{99m}TcO_2$) [15]. In general, RCP testing performed using thin layer chromatography (TLC) method with organic solvent as mobile phases and TLC silica paper or instant TLC as static phases [16].

The RCP of ^{99m}Tc-MIBI method used according to kit manufacturer is usingaluminum oxide plate type-T as static phase and ethanol absolute (99.8%) as mobile phase. This method separates$^{99m}TcO_4^-$with Rf of 0.4 – 0.6 and$^{99m}TcO_2$with Rf of 0.0 from^{99m}Tc-MIBI with Rf of 1.0.However, the time required to perform this method is about 2.0 hours [16,17]. Therefore, in 2016 Maskur *et,al*. conducted research a new method which was expected to be much faster using extraction testing method where organic and water phases were measured using dose calibrator (DC) [18]. However, DC is not very stable when used for measuring a sample with a small volume. Therefore, in this research was studied the comparison the measurement result of % RCP ^{99m}Tc-MIBI with extraction method which measured using two types of tools, dose calibrator (DC) and gamma counter (GC). The purpose of this study is to find out whether the testing of RCP ^{99m}Tc-MIBI using extraction testing method where their phases measured usingDC and GC is not differ significantly compared to that of TLC method.

METHODS

Material

Materials used including MIBI kit (PT. Kimia Farma), ^{99m}Tc radionuclide (retrieved from an in-house produced from ^{99}Mo/^{99m}Tc generator, Center for Radioisotope and Radiopharmaceutical Technology), ethanol 99.9%, sodium chloride 0.9%, chloroform 99.8%, and 1x11 cm strips of TLC aluminum cellulose (Merck).

Equipment

Equipment used including stopwatch, cylindrical glass chamber for chromatography, pipettes and its tips (Eppendorf), 2 mLmicro tubes (Isolab), dose calibrator (AtomLab 300 Biodex), gamma counter (Caprac-t).

Radiolabeling

MIBI kit is labeled with ^{99m}Tc to result in^{99m}Tc-MIBI. A vial of MIBI kit was placed in lead containers,^{99m}Tc in the form of sodium pertechnetate ^{99m}Tc obtained from ^{99}Mo/^{99m}Tc generator then added using a syringe (~ 100 mCi)in 3 mL. The mixture was shaken and heated in a boiling water bath for 10 minutes followed by cooling at room temperature before analysis [19].

Radiochemical Purity Testing

Standard Method

Determination of RCP of^{99m}Tc-MIBI is performed by ascending TLC with aluminum cellulose as static phase and ethanol absolute as mobile phase. An aliquot amount of the ^{99m}Tc-MIBI (2 µL) was pipetted and spottedon the base line (2 cm from the lower end) of aluminum cellulose strips. The strips were developed in achromatography chamber containing approximately 1 cm high layer of absolute ethanol until reaching ~ 0.5 cm from the top of strips (for approximately 2.0 hours). The strips were removed from the chamber, dried in the air and then cut (1 cm/ portion). Each portion of the strips was counted using GC. Radioactivity spots were identified according their Rfs value. Free the $^{99m}TcO_4^-$ migrates with the solvent to the give an Rf of 0.4 – 0.6, $^{99m}TcO_2$ remain on the starting line to give an Rf of 0.0, and the ^{99m}Tc-MIBI complex migrates with the solvent front to give an Rf of 1.0. The total impurities($^{99m}TcO_4^-$ and $^{99m}TcO_2$) should not be higher than 10% of the total activity [14,19]. The % RCP of ^{99m}Tc-MIBI can be calculated by Equation 1:

$$\%RCP\ ^{99m}Tc-MIBI = \frac{Total\ counts\ of\ ^{99m}Tc-MIBI}{Total\ counts\ of\ ^{99m}Tc-MIBI + {}^{99m}TcO_4^-} \quad (1)$$

Extraction Method

The RCP of ^{99m}Tc-MIBI test using extraction method began by separating the radiochemical components in available sample. Separation was performed using by 3.0 mL of 0.9% sodium chloride as hydrophilic phase and 3.1 mL of chloroform aslipophilic phase. Aliquot amount (0.1 mL) was added into the mixture solvent,and then stirred using vortex with medium strength, let to stand until the solvent mixture separated into two phases. The lipophilic ^{99m}Tc-MIBI will be extracted into the lipophilic phase (lower layer), whereas the hydrophilic compounds, $^{99m}TcO_4^-$ and $^{99m}TcO_2$will be extracted into the hydrophilic phase (top layer) [18]. Aliquot amount of each phases (0.25 mL) was withdrawn and measured its radioactivity using DC and GC.

The Quantification Limit Test (LOQ)

The procedure is performed according to the extraction method with variation of sample volume. Each phase is taken 1, 5, 10, 50, 100, and 250 µL. Then sample is measured using DC and GC tools.

Data Analysis

The retrieved data (DC and GC; TLC and GC) were compared by using two sample assuming equal variance to test for significant differences between two methods with assumption that both samples had the same variance. The t-test of two independent samples with the same variance is calculated by the Equation 2 [20, 21].

$$t = \frac{x_1 - x_2}{\sqrt{\left(\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2}\right)\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}} \quad (2)$$

Where

t = t-value,
x1 = average data of sample 1,
x_2 = average data of sample 2,
S_1 = sample varian 1,
S_2 = sample varian 2,
n_1 = sample size 1, and
n_2 = sample size 2

The percentage of RCP of the ^{99m}Tc-MIBI with the above-mentioned methods was analysed by using t-test. The null hypothesis (H0) is assumed to be true if % RCP ^{99m}Tc-MIBI between two methods is not significantly different. The difference between both methods are not significant if the t-value of t-test statistic is less than 2.10 with significance level (alpha) = 0.05 with number of degrees freedom (df) = 18.

RESULTS AND DISCUSSION

The standard method for determine of RCP ^{99m}Tc-MIBI is by using TLC where aluminum cellulose strips used as static phase and ethanol absolute as mobile phase. The resulted chromatogram from this test is shown in Fig. 1.

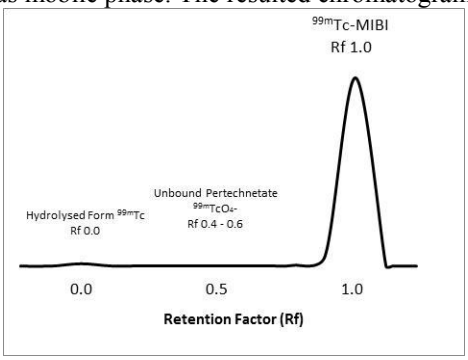

FIGURE 1.Chromatogram of ^{99m}Tc-MIBI (TLC:Aluminum cellulose strip/ ethanol absolute as eluent)

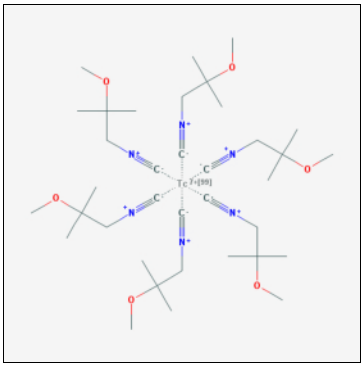

FIGURE 2. Chemical Structure of ^{99m}Tc-MIBI [13]

The results of the RCP test using TLC method show a good separation between the ^{99m}Tc-MIBI complex and its impurities. The ^{99m}Tc-MIBI is separated on Rf 1.0 because ethanol absolute is a semi polar mobile phase with dielectric constant of about 21.2 [22]. This property can dissolve both polar and nonpolar molecules (lipophilic). It can be seen in Fig. 2 that molecular structure of MIBI has many alkyl hydrocarbon chains. Not only that, the electronegativity of ^{99m}Tc-MIBI molecule bond is also very small as the alkyl group (-CH3) χ = 0.4, the carbon-carbon bond (C-C) χ = 0.0, as well as the carbon-nitrogen bond (C-N) χ = 0.4. Low electronegativity makes the ^{99m}Tc-MIBI as nonpolar compound to dissolve in the semi polar and nonpolar solvents [23]. The impurities of $^{99m}TcO_2$ stay at Rf of 0.0 and $^{99m}TcO_4$- Rf of 0.4 – 0.6.

In addition to TLC method, the extraction method might also be used to separate ^{99m}Tc-MIBI where impurities. MIBI will readily dissolve in lipophilic solvents such as chloroform with dielectric constant 4.8 whereas the hydrophilic$^{99m}TcO_4^-$ and $^{99m}TcO_2$ impurities will readily dissolve on hydrophilic solvent. The hydrophilic solvent used is 0.9% sodium chloride with constant dielectricity of 73.3 [24]. However, extraction method also has someweaknesses, particularly the sensitivity of the measuring/ tool used. The results of ^{99m}Tc-MIBI radiochemical purity measurement using DC and GC with variation of sample volume are shown in Fig. 3.

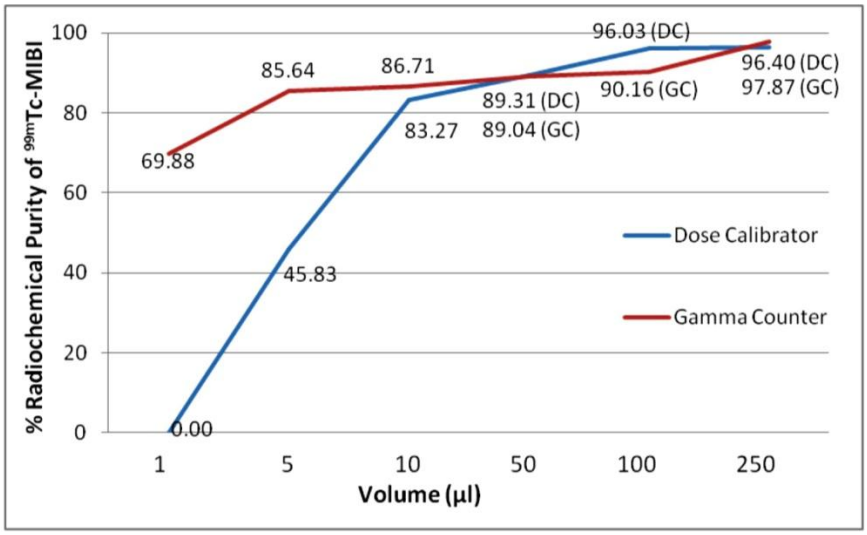

FIGURE 3. Percentage of RCP ^{99m}Tc-MIBI in various sample volume

It can be seen in Figure 3 that the percentage of RCP of ^{99m}Tc-MIBI varies depending on the sample volume being measured. In the DC tool the volume of sample 1 µL cannot be measured so the result becomes 0.0%, however as the sensitivity of GC tool is better than to that of DC, the measurement the same sample using GC gave the percentage of RCP of 69.88%. The percentage of RCP value of ^{99m}Tc-MIBI is still below 90% with the sample volume of 5 µL to 50 µL on DC and GC. The percentage of RCP starts to stabilise on larger sample volume of 100

μL with over than 90%. The maximum value of percentage of RCP is shown in the largest sample volume of 250 μL with percentage of RCP of 96.40% and97.87% using DC and GC tools respectively.

The DC is a device used for measuring radioactivity of a radionuclide with a measurement unit of Curie (Ci) or Becquerel (Bq). The wall of the detector is connected to the negative pole of the voltage source as the cathode while the wire in the center of the tube is connected to the positive pole as anode [25]. However, when measuring the RCP of radioactivity the values that appear on the display should be deducted with the background. Therefore, a low radioactive activity cannot be well read by DC device [26]. The GC is a device used for measuring gamma energy decay from radionuclide resulted in a count per minute (cpm) or count per second (cps). This tool is designed with high sensitivity using NaTI crystals. The instrument efficiency for detecting gamma rays depends on the thickness of the crystals. GC can measure gamma energy with low activity in a small amount of samples [27].

As a sample with 1 μL the DC did not show reading, therefore the percentage of RCP of ^{99m}Tc-MIBI cannot be measured due to low activity. On the other hand the GC shows the reading for the same sample therefore the percentage of this sample might be calculated. This phenomenon occurs because of the value of energy conversion curie is smaller than to that of cps. One μCi equals 37,000 cps. Based on comparison data of radioactivity measurement using DC and GC, it can be concluded that the biggest sample volume show more accurate percentage of RCP of ^{99m}Tc-MIBI. Thus, the recommended sample volume for determine percentage of RCP of ^{99m}Tc-MIBI is 250 μL for both DC and GC devices.

Further validation of test result data using TLC, DC and GC methods are shown in Table 1 and Table2.

TABLE 1. The percentage of RCP of ^{99m}Tc-MIBI using various testing method

	Percentage of RCP ^{99m}Tc-MIBI (%)		
	TLC	**Dose calibrator**	**Gamma counter**
1	98.02	98.09	97.40
2	98.25	97.54	97.58
3	98.54	97.24	97.60
4	97.50	97.43	98.82
5	97.36	97.45	98.80
6	97.25	97.48	98.63
7	98.28	97.60	97.05
8	98.01	96.78	98.19
9	97.17	96.57	97.62
10	98.02	98.29	97.85
Mean	97.84	97.45	97.95
%RSD	0.49%	0.53%	0.64%
% Accuracy		99.60%	100.12%
Time consuming	171.90 ± 3.90 minutes	22.10 ± 1.85 minutes	22.20 ± 1.55 minutes

TABLE 2. Analysis data of Comparison extraction method(DC and GC), comparison extraction (GC) and TLC methods

Parameter	**Comparison extraction method (dose calibrator and gamma counter)**	**Comparison extraction and TLC methods (gamma counter and TLC)**
df	18	18
Alpha (α)	0.05	0.05
p-value	0.06	0.65
t-critical-value	2.10	2.10
t-value	1.98	0.46

Table 1 represents the average of percentageof RCPof ^{99m}Tc-MIBI using TLC, DC and GC methods of 97.84 ± 0.49%, 97.45± 0.53%, and 97.95 ± 0.64% respectively. The results of percentage of RCP of ^{99m}Tc-MIBI for three methods show that the percentage of relative standard deviation (% RSD) less than 1% which confirms that the measurement results have a good precision. The percent accuracy of the extraction method using a dose calibrator is

99.60%, using a counter count of 100.12%. This value indicates that the gamma counter method is more accurate than the dose calibrator method.In addition, the most efficient test time is using the dose calibrator extraction method or gamma counter with 22.10 ± 1.85 minutes and 22.20 ± 1.55 minutes respectively.

To determine whether the data differ significantly or not can be seen in the following hypothesis. H0 is accepted if p-value is greater than α of 0.05 and t-value less than t-critical value of 2.10. In addition at Table 2, based on this experiment, p-value of DC and GC (0.06) and GC and TLC (0.65), both of p-value isgreater than alpha of 0.05.Since data comparison DC and GC with the t-value of 1.98 while data comparison between TLC and GC with the t-value of 0.46, that data is less than t-critical value 2.10, so the H0 hypothesis is accepted. That statistically calculation can prove that the data distribution of percentage of RCP of ^{99m}Tc-MIBIdid not differ significantlyby using TLC, DC and GC methods.

CONCLUSION

Generally, all three methods can be performed to determine the radiochemical purity of ^{99m}Tc-MIBI. However, to obtain good results, the measurement using dose calibrator and gamma counter for extraction methods must use a minimum sample volume 250 µL.

Comparison of extraction with two different measurement tools i.e. dose calibrator and gamma counter were not significantly different based on statistical calculation with t-value of 1.98. In addition, comparison between thin layer chromatography and gamma counter were not also significantly different with t-value of 0.46. However, the best method to determine radiochemical purity of ^{99m}Tc-MIBI is using the gamma counter extraction method with a time of 22.20 ± 1.55 minutes with 100.12% percent accuracy.

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