VALIDATION OF SIMPLE UV-VIS SPECTROPHOTOMETRY METHOD BASED ON ICH Q2(R1) GUIDELINE FOR THE ANALYSIS OF SODIUM HYPOCHLORITE USING RHODAMINE B

VALIDASI METODE SPEKTROFOTOMETRI UV-VIS BERDASARKAN PEDOMAN ICH Q2(R1) UNTUK ANALISA NATRIUM HIPOKLORIT MENGGUNAKAN RHODAMINE B

Miftakul Munir, Siska Febriana, Witarti
Center for Radioisotop and Radiopharmaceuticals Technology, National Nuclear Energy Agency (BATAN), Puspiptek Area Serpong, Tangerang Selatan, Indonesia 15314
Email: miftakul@batan.go.id, miftahmun@gmail.com

ABSTRACT

Sodium hypochlorite (NaOCl) has many functions and one of them is as an oxidizing agent in $^{99}Mo/^{99m}Tc$ generator based on zirconium-based material (ZBM). However, the NaOCl has cytotoxic effect which can harm human body. Hence, there is a need for analytical procedure for its determination. This study describes the validation parameters given by International Conference on Harmonization (ICH) Q2(R1) to ensure that the UV-Vis spectrophotometric method using rhodamine B is suitable for determination of NaOCl concentration. This method was validated in respect of linearity, specificity, and sensitivity. Whereas, the parameter of accuracy and precision need to be developed to meet the acceptable criteria. The results in a range of linearity, coefficient of correlation, and limit of quantification are 0.2–4.8 mg L^{-1}, 0.996 mg L^{-1}, and 0.2 mg L^{-1} respectively.

Keywords: validation, NaOCl, rhodamine B, ICH.

ABSTRAK

Natrium hipoklorit (NaOCl) mempunyai banyak fungsi, salah satunya adalah sebagai oksidator dalam generator $^{99}Mo/^{99m}Tc$ berbasis zirconium-based material *(ZBM). Akan tetapi, NaOCl memiliki efek sitotoksik yang dapat membahayakan tubuh manusia. Oleh karena itu, diperlukan metode analisis untuk mengukur kadar NaOCl. Studi ini menjelaskan berbagai parameter validasi yang diberikan oleh* International Conference on Harmonisation *(ICH) Q2(R1) untuk memastikan bahwa metode spektrofotometri UV-Vis menggunakan rhodamine B sesuai untuk mengukur konsentrasi NaOCl. Metode ini divalidasi dan memenuhi parameter linieritas, spesifisitas, dan sensitivitas. Sementara itu, parameter akurasi dan presisi masih perlu dikembangkan lebih lanjut untuk memenuhi kriteria yang dipersyaratkan. Hasil pengukuran parameter rentang linearitas, koefisien korelasi, dan batas kuantifikasi masing-masing adalah 0,2–4,8 mg L^{-1}, 0,996 mg L^{-1}, and 0,2 mg L^{-1}.*

***Kata kunci**: validasi, NaOCl, rhodamine B, ICH*

1. INTRODUCTION

Sodium hypochlorite (NaOCl) is a chemical compound which commonly used as bleaching agent and disinfectan in many fields (Braitt et al., 2013; Pasha & Narayana, 2007). In dentistry, the NaOCl is routinely used as endodontic irigant. Another application of the chemical is as reagent in analytical chemistry (Abdulrahman & Basavaiah, 2012) and oxydator in $^{99}Mo/^{99m}Tc$ generator based on zirconium-based material (ZBM), which can enhance the yield of 99mTc (Saptiama et al., 2015).

On the other hands, the NaOCl has cytotoxic effect which can harm human body (MS, 2016; Zhu et al., 2013). There is no official guideline for human exposure limitation of NaOCl on medical practise, however, based on WHO guideline, the limitation in drinking water is 0.2–1 mg L^{-1} (Guedes-silva et al., 2016). The use of NaOCl in parenteral pharmaceuticals, e.g. $^{99}Mo/^{99m}Tc$ generator based on ZBM, must be strictly controlled due to its toxicity. Therefore, there is a need for analytical procedure for determination of NaOCl concentration. Pasha and Narayana (2007) reported a facile UV-Vis spectrophotometric method for determination of NaOCl concentration using rhodamine B, which meets Beer's law in the range of 0.1–4.0

µg mL^{-1} of NaOCl. UV-Vis spectrophotometry is an inexpensive and adequate analytical method compared to the other method (Dhole et al., 2012; Thiruvengadam, Ramadoss, & Vellaichamy, 2013).

The spectrophotometric method for determination of NaOCl concentration using rhodamine B has to be validated because it will be applied in routine analysis especially in pharmaceuticals (Ajay & Rohit, 2012). The objective of the validation is to ensure that an analytical method is suitable for its intended purpose (Silva-Buzanello et al., 2015). The guideline from the International Conference on Harmonization ICH-Q2(R1) clearly describes validation characteristics for consideration in the validation of analytical method (Li, Igne, Drennen, & Anderson, 2016; Navas et al., 2013; Pein, Eckert, Preis, & Breitkreutz, 2013). For the analysis of a impurities, the recommended following characteristics are linearity, range, quantification limit (LOD), repeatability, intermediate precision, accuracy, and specificity (Branch, 2005).

In the previous study, we have studied the application of spectrophotometric method for determination of NaOCl concentration in ^{99m}Tc eluate of ^{99}Mo/^{99m}Tc generator based on ZBM (Munir, Witarti, & Febriana, 2016). ^{99}Mo/^{99m}Tc generator is a generator which produces a $^{99m}TcO_4^-$ solution for medical procedures. $^{99m}TcO_4^-$ solution is the most widely used radionuclide solution in nuclear medicine. More than fifteen radiopharmaceutical kits is radiolabelled by a $^{99m}TcO_4^-$ solution (Osso Jr et al., 2012; Pillai, Dash, Knapp, 2014).

This study focused on demonstrating that UV-Vis spectrophotometric method using rhodamine B is suitable and adequate for determination of NaOCl concentration by validating the method based on ICH guideline Q2(R1). The validation characteristics of the guideline was adapted and modified based on the use of NaOCl in ^{99}Mo/^{99m}Tc generator based on ZBM.

2. METHOD

The following chemical compound were used as they were received from commercial sources, such as sodium tiosulphate ($Na_2S_2O_3$, Sigma Aldrich), potassium iodate (KIO_3, Merck), starch indicator 1% (Sigma Aldrich), rhodamine B (Sigma Aldrich), solution of NaOCl (Sigma Aldrich), potassium iodide (KI, Merck), hydrochloric acid (HCl, Merck), and sodium acetate ($NaCH_3COO$, Sigma Aldrich). Eluate of ^{99m}Tc from ^{99}Mo/^{99m}Tc generator based on ZBM was obtained from Laboratory of Center for Radioisotope and Radiopharmaceutical Technology, National Nuclear Energy Agency (BATAN).

A UV-Visible spectrophotometer *Perkin Elmer Lambda 45* equipped with 1 cm matched quartz cell was used for all absorbance measurements.

The linearity was investigated using standard solution of NaOCl in six different concentration levels, ranging from 0.2 mgL^{-1} to 4.8 mgL^{-1}. The standard solutions were transferred into 10 mL calibrated flasks containing 1 mL of 2 mol L^{-1} HCl and 1 mL of 2% KI. The mixtures were shaken until a yellow color appeared. A 2 mL of 1 mol L^{-1} NaCH3COO and 3 mL distilled water were added to it, followed by addition of 0.5 mL of 0.01% rhodamine B solution. The mixture was shaken for three minutes, then diluted to 10 mL with distilled water and mixed well. Measurements of absorbance of the resulting solutions were carried out using UV-Visible spectrophotometer at 555 nm (Munir et al., 2016; Pasha & Narayana, 2007).

Determination of the LOQ was carried out using standard deviation (*s*), calculated from seven blank samples, and the slope value (*b*) was obtained from calibration curve according to Equation 1. A blank sample was prepared by replacing NaOCl standard solution with distilled water (Silva-Buzanello et al., 2015).

$$LOQ = \frac{3 \times S}{b} \quad \text{..... (1)}$$

The investigation of the repeatability (different assays on the same day) and the intermediate precision (assays on different days) was conducted using three concentrations level of NaOCl (0.2, 3.2, and 4.8 mg L^{-1}) and three replications for each sample. The precision was calculated by the relative standard deviation of the resulting investigation (Silva-Buzanello et al., 2015).

The accuracy of analytical method was determined by analyzing three concentrations level of NaOCl, which correspond to minimum, medium, and maximum concentration from the calibration curve. The assays were conducted in triplicate on two different days and calculated by using calibration curve (Silva-Buzanello et al., 2015).

The specificity was determined by conducting interference study of the sodium chloride to the analytical result. The interference study was performed by replacing distilled water with saline solution (sodium chloride 0.9%) in sample preparation. Saline solution is eluent for $^{99}Mo/^{99m}Tc$ generator, therefore sodium chloride was used in the interference study.

3. RESULT AND DISCUSSION

The absorbance curve of rhodamine B in acid environment is presented in Figure 1.

Figure 1 shows the absorbance curve of rhodamine B in presence of potasium iodide (KI) without NaOCl. The addition of NaOCl will reduce the peak absorbance of rhodamine B. This is due to NaOCl liberates Iodine (I2) which fades the color of rhodamine B. The color reduction of rhodamine B is proportional to the rising of NaOCl concentration (Pasha & Narayana, 2007). Therefore, NaOCl concentration can be determined using calibration curve of its concentration against an absorbance of rhodamine B color. Figure 2 presents the mechanism of the color reduction reaction.

The absorbance of rhodamine B is influenced by the acidity of solution (Munir et al., 2016), this buffer acetate was added to maintain the acidity of solution (Pasha & Narayana, 2007).

Sample preparation time may be varied for each process. Hence, there is a need to ascertain that the time variation has no influence to NaOCl concentration measurement. The stability of rhodamine B color in the presence of NaOCl has been studied and presented in Figure 3.

Based on graph in Figure 3, the color of rhodamine B is stable until sixty minutes. The more stable the color, the more precise the measurement. The stability time is enough for the time variation of sample preparation and there is no interference to the NaOCl concentration measurement. Figure 4 below presents the calibration curve of NaOCl concentration series.

Figure 4 shows the calibration curve of absorbance against concentration of NaOCl, which meets Beer's law from 0.2 mg L^{-1} to 4.8 mg L^{-1} of NaOCl. The range of the linearity is wider than previous developed method. Pasha and Narayana (2007) reported the linearity was

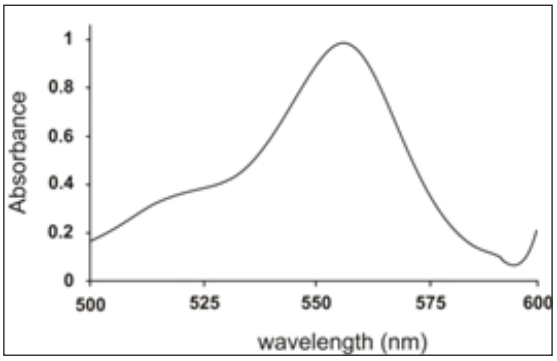
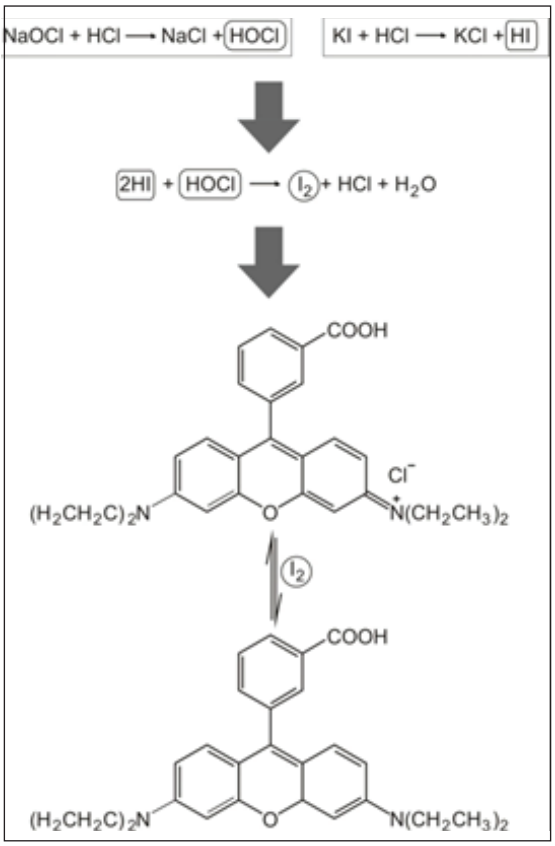


Figure 1. Absorbance Curve of Rhodamine B in Acid Solution (pH 4)



Source: Pasha & Narayana, 2007

Figure 2. Scheme Mechanism of Rhodamine B Color Reduction

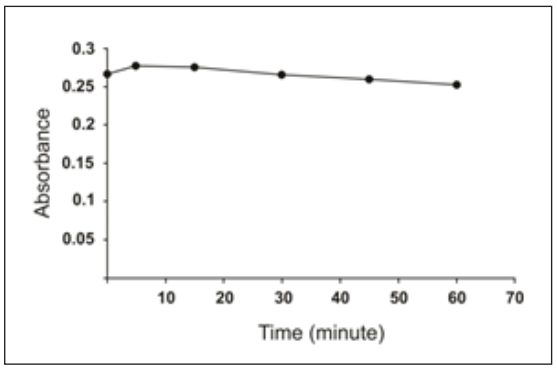


Figure 3. The Stability of Rhodamine B Color in the Presence of NaOCl

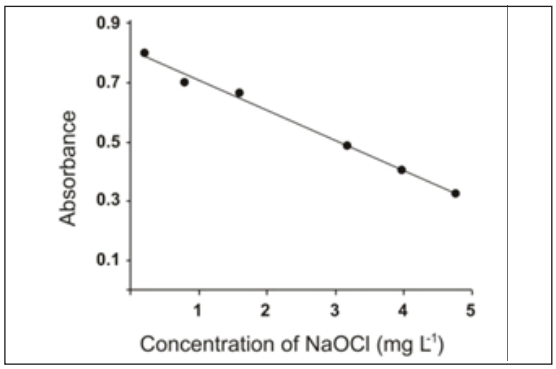


Figure 4. Calibration Curve of NaOCl Concentrations

in the range of 0.1–4.0 mg L^{-1} (Abdulrahman & Basavaiah, 2012), whereas in the previous study, we reported the linearity was in the range of 0.19–2.25 mg L^{-1} (Pillai, Dash, & Knapp, 2014). Actually, there is no guideline which shows a limitation of NaOCl concentration in parenteral pharmaceuticals. However, some governments regulate NaOCl limitation in drinking water of 2–4 mg L^{-1}, so the linearity of method is sufficient for NaOCl concentration measurement.

The resulted coefficient correlation of calibration curve is 0.996, this value is higher than previous developed method. Pasha and Narayana (2007) as well as our previous study obtained coefficient correlation of 0.995 (Pillai et al., 2014). The difference between both the present and previous one is not significant, and the coefficient correlation of present method is sufficient for NaOCl concentration measurement. The Official Regulatory Affair (ORA) Laboratory of Food and Drug Administration (FDA) requires the coefficient correlation should be greater than or equal to 0.995 (*APPENDIX I – ORA validation and verification guidance for human drug analytical methods,* 2009). The equation of calibration curve and limit of quantification is y = 0.812–0.100x and 0.2 mg L^{-1} respectively. The precision and the accuracy of the method have been calculated and presented in Table 1.

Table 1 shows the measurement of the precision and the accuracy of the method. Precision values for the repeatability (intraday precision) and the intermediate precision (interday precision) are presented by relative standard deviation (RSD). The precision values are varied from 3.18% to 8.37%. There is a need to develop the method for obtaining the lower RSD. Kumar and Venkateshwarlu (2013) developed spectrophotometric method using rhodamine B and obtained RSD lower 2%. Whereas Tirupathi and Venkateshwarlu (2015a; 2015b), in their developed method using rhodamine B, obtained RSD lower than 1%. The ORA Laboratory of FDA gives the RSD limit value less than or equal to 2.0%, unless otherwise specified (*APPENDIX I – ORA validation and verification guidance for human drug analytical methods,* 2009).

The accuracy values are presented by recovery. The recoveries from low, middle, and

Table 1. The Precision and the Accuracy of the Method

NaOCl concentration (µg mL^{-1})	Precision			Accuracy	
	1st day RSD (%)	2nd day RSD (%)	Inter-day RSD (%)	Concentration found (µg mL^{-1})	Recovery n=6 (%)
0.8	5.61	8.35	8.25	0.97	123
3.2	3.18	3.17	3.23	3.19	101
4.8	8.01	5.63	8.37	4.83	102

high concentration are 123%, 101%, and 102% respectively. Generally, acceptable criteria is between 70–120%, depends on the sample preparation and analytical procedure (Silva-Buzanello et al., 2015). The recoveries from middle and high concentration are good, but the one in low concentration is too high (123%). The interference study of the method using sodium chloride shows there is no significant result of NaOCl measurement.

In this study, we also conducted the analysis of NaOCl concentration in the eluate of ^{99}Mo/^{99m}Tc generator based on ZBM using the present method. The result of measurement is 3.56 mg L^{-1} with RSD of 3.65%. This value is lower than the limit value in daily drinking water, hence the NaOCl concentration in the eluate was considered to be safe for human body (WHO, 1996).

4. CONCLUSION

The UV-Vis is a spectrophotometry method for determination of NaOCl using rhodamine B and validated based on ICH-Q2(R1) in respect of linearity, specificity, and sensitivity. Whereas, the parameter of accuracy and precision is need to be developed to meet the acceptable criteria. The method presented a range of linearity, coefficient of correlation, and limit of quantification of 0.2–4.8 mg L^{-1}, 0.996 mg L^{-1}, and 0.2 mg L^{-1} respectively.

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