Development and Validation of Spectrophotometry UV-Vis Method for Determination of Primaquine and Chloroquine in Liposome Dosage Form

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RESEARCH ARTICLE

Development and Validation of Spectrophotometry UV-Vis Method for Determination of Primaquine and Chloroquine in Liposome Dosage Form

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ABSTRACT:
Development and validation of UV-Vis Spectrophotometry method for simultaneous determination of primaquine and chloroquine in liposome dosage form has been carried out. The method was tested for selectivity, linearity, accuracy, and precision. A phosphate buffer solution pH 7.4 was used as a solvent and observations were made at wavelengths of 220 and 260 nm for simultaneous equations method. The selectivity results of primaquine and chloroquine showed no interference from liposome. Linearity result (*r* value) of the simultaneous equation method was 0.9998 with the value of Vxo less than 0.5% for both primaquine and chloroquine in the concentration range of 2-10 mg/L. Accuracy was done using spiked placebo method and obtained data analyzed using simultaneous equation method. Persentage recovery of primaquine was 89-97% and 79 - 108% for chloroquine. The intra- and interday precision of primaquine were 1.72 and 2.57%, respectively. Whereas the intra- and interday precision of chloroquine were 6.93 and 8.77, respectively. Further observation using chromatography method need to be done to have better accuracy results for both substances.

KEYWORDS: Primaquine, Choloroquine, Liposome, Validation, UV-Vis Spectrophotometry

INTRODUCTION:

Malaria is an infectious disease with a high mortality rate. According to the World Malaria Report, it was reported that malaria had attacked 106 countries, and generally occurred in tropical and sub-tropical regions including Indonesia. [1, 2, 3] To actualize Malaria Free Asia Pacific in 2030, the development of malaria drug formulations is carried out to make it able to deliver the drug to the desired target receptor. [4] Primaquine and chloroquine are combinations of drugs that can improve the effectiveness of malaria treatment therapy. [5, 6] Primaquine is the primary drug used to prevent recurrence of malaria caused by Plasmodium vivax and Plasmodium ovale. The combination of primaquine and chloroquine is very effective in eliminating Plasmodium vivax from the blood so that it can increase the potential of primaquine. [7]

The primaquine and chloroquine preparations are being developed in the form of liposome preparations. [8] This drug combination is expected to increase the effectiveness of the drug through altering the pharmacokinetic profile of drugs that have high toxicity and low bioavailability, such as primaquine. [9, 10, 11, 12, 13]

To support the development of a combination formulation of primaquine and chloroquine, a suitable analysis method is needed. This analytical method is needed to ensure that the quality of the drugs in liposome dosage form meet the specifications.

For the analysis of primaquine and chloroquine in liposome, the UV-Vis spectrophotometry method was developed. The UV-Vis spectrophotometry method was chosen based on the consideration that matrix could not interfere the absorbance readings of primaquine and chloroquine. In addition, the UV-Vis spectrophotometry method is relatively efficient compared to the chromatographic method.

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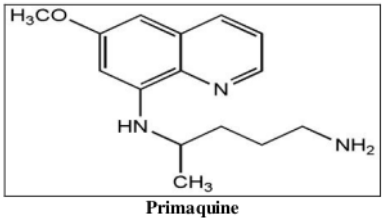
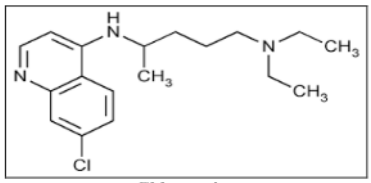


Primaquine



Chloroquine

Figure 1 Chemical structure of Primaquine and Chloroquine

MATERIAL AND METHODS:

Chemicals and reagents:

Primaquine and chloroquine were procured from Indonesian Drug and Food Control Agency. Primaquine and chloroquine in liposom dosage form were made in Pharmaceutics Laboratory (Universitas Airlangga, Indonesia). Sodium phosphate dibasic and monobasic were purchased from Merck. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Instrumentation:

The proposed work was carried out on a Shimadzu UV-Visible spectrophotometer (model UV-CARY 60), with a 1 cm quartz matched cell. Mettler Tolledo Analytical Balance was used for weighing analytes.

Selection of Solvents:

Phosphate Buffer Saline pH 7.4 (PBS) was selected as solvents due to further in vitro purposes.

Preparation of Standard Stock Solutions of Primaquine and Chloroquine:

Stock Solution of Primaquine:

Weighed quantitavely 50 mg of primaquine standard then dissolved in 25 mL phosphate buffer saline (PBS) to obtain a standard stock solution of Primaquine (2000 mg/L).

Working Standard Solution of Primaquine:

Stock solution of Primaquine (2000 mg/L) was diluted with PBS to get five concentrations of working standard solution with the range of 2 to 10 mg/L.

Stock Solution of Chloroquine:

Weighed quantitavely 50 mg of chloroquine standard then dissolved in 25 mL phosphate buffer saline (PBS) to obtain a standard stock solution of Chloroquine (2000 mg / L).

Working Standard Solution of Chloroquine:

Working solution of chloroquine was made by diluting chloroquine stock solution to obtained a range concentration of 2 to 10 mg/L

Determination of maximum wavelength (λ_{max}) of primaquine and chloroquine :

The maximum wavelength of UV-Vis spectrophotometry was carried out by scanning the standard solution of primaquine and chloroquine in the wavelength range of 200-500 nm. The selected wavelength was determined based on the highest absorptions of each standard solution which was not disturbed by matrices or impurities.

Validation Method :

Specificity

Specificity tests were carried out by scanning primaquine, chloroquine and matrices on selected wavelengths. Specificity was determined based on the absorbance of primaquine and chloroquine without interferences from matrices and impurities.

Linearity

Linearity was made by scanning the absorbance of working standard solution of primaquine and chloroquine with a concentration between 2-10 mg/L. Linearity was determined by linear regression equation and r value.

Accuracy and Precision

Accuracy tests were carried out using three different concentrations of primaquine and chloroquine standard solutions. Accuracy was done by the spiked-placebo method. For each concentration replication was carried out in triplicates. Accuracy was expressed as percentage recovery.

Precision was obtained by calculating the relative standard deviation (RSD) value from three different concentrations of each component. Precision was done in triplicates and determined as Coeeficient Variation (CV).

RESULTS AND DISCUSSIONS:

Selected maximum wavelengths of primaquine and chloroquine was shown in Figure 2. Overlay of primaquine, chloroquine and matrices (liposome) showed that liposomes did not interfere the absorbance reading of primaquine and chloroquine at selected wavelengths.

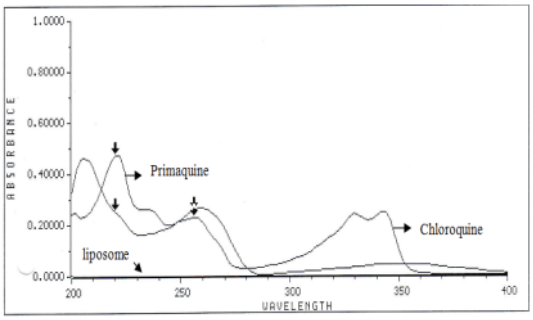


Figure 2. Overlay spectra of Primaquine, Chloroquine, and liposome. Wavelength of 220 nm and 260 nm were selected for further analysis of Primaquine and Chloroquine.

Linearity of Primaquine and Chloroquine:

Absorbance of primaquine and chloroquine was carried out at wavelengths of 220 nm and 260 nm. Absorbance readings were carried out in a concentration range from 2 to 8 mg/L. The linearity of primaquine and chloroquine were calculated using simultaneous equation method and presented in Table 1.

Table 1: Linear regresion of Primaquine and Chloroquine:

Parameters	Primaquine		Chloroquine	
	220 nm	260 nm	220 nm	260 nm
Slope	0.065	0.082	0.088	0.040
Intercept	- 0.016	- 0.003	-0.013	- 0.005
R^2 value	0.999	0.999	0.987	0.983

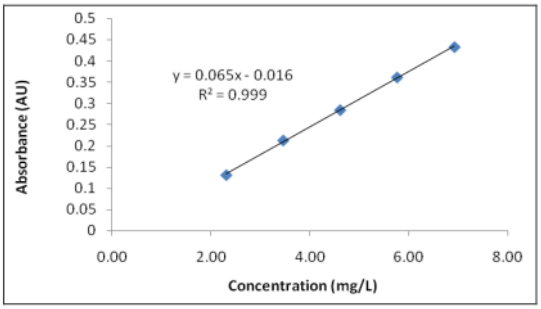


Figure 3. Calibration Curve of Primaquine at wavelength of 220 nm.

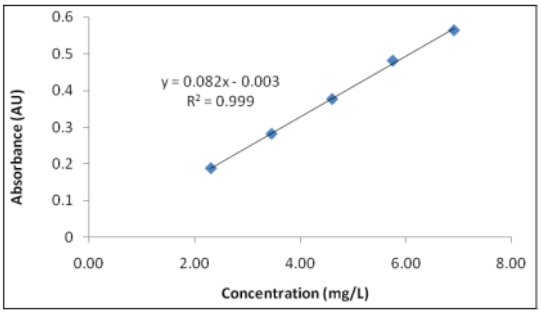


Figure 4. Calibration Curve of Primaquine at wavelength of 260 nm.

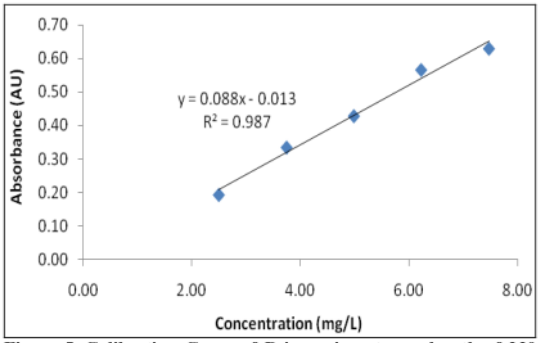


Figure 5. Calibration Curve of Primaquine at wavelength of 220 nm.

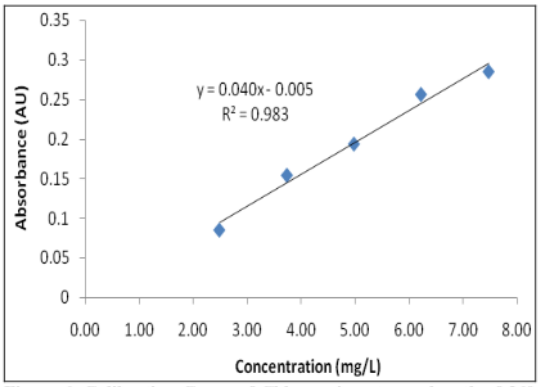


Figure 6. Calibration Curve of Chloroquine at wavelength of 260 nm.

Accuracy Test:

Accuracy test was done by using spiked-placebo method. Standard of primaquine and chloroquine were added to the matrices to make concentration of 80%, 90% and 100% of label claimed. Accuracy test was carried out in triplicates and the calculation of accuracy was done using simultaneous equation method. Accuracy results of primaquine and chloroquine are presented Table 2 and Table 3.

Table 2: Accuracy result of Primaquine calculated using simultaneous equation method

Concentration of label claimed	Primaquine Added (mg/L)	Primaquine Obtained (mg/L)	% Recovery
80%	3.46	3.08	91.02
		3.15	89.00
		3.15	91.05
90%	5.76	5.42	94.10
		5.38	93.40
		5.32	92.36
100%	6.92	6.72	97.18
		6.49	93.83
		6.53	94.40

Table 3: Accuracy result of Chloroquine calculated using simultaneous equation method:

Concentration of label claimed	Chloroquine Added (mg/L)	Chloroquine Obtained (mg/L)	% Recovery
80%	3.74	2.96	79.24
		3.08	81.41
		3.04	82.38
90%	6.22	6.09	97.91
		5.77	92.77
		5.78	92.93
100%	7.47	8.05	107.70
		7.92	106.09
		7.96	106.56

The accuracy test results of primaquine and chloroquine calculated using the simultaneous equation method showed a percent recovery value of 89 to 97%, while for chloroquine the percent recovery was 79 to 108%. According to AOAC, the accuracy test for active compounds with a level of 0.001%, the percentage of recovery allowed is 90-107%. Since the accuracy results of primaquine and chloroquine did not meet the requirements, it is necessary to do an experiment by conducting a chromatographic method. One of reason that might cause the accuracy did not fulfill the requirement was the large differences in absorptivity at different wavelengths.

Precision:

Precision was determined as intra-day and inter-day. For intra-day, primaquine and chloroquine were analyzed three times at the same day. Whereas for inter-day, Primachloroquine and Chloroquine was analyzed at different days. The CV of intra-day precision were 1.72 % and 6.93% for Primaquine and Chloroquine, respectively. While the inter-day precision for Primaquine and Chloroquine were 2.57% and 8.77%, respectively.

CONCLUSION:

Spectrophotometry UV-Vis method can be used for simultaneous analysis of primaquine and chloroquine in lipid dosage form. Further development of chromatographic method need to be developed to get better results than spectrophotometric method.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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