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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF CHLOROQUINE AND PRIMAQUINE IN ITS BULK AND PHARMACEUTICAL DOSAGE FORMS

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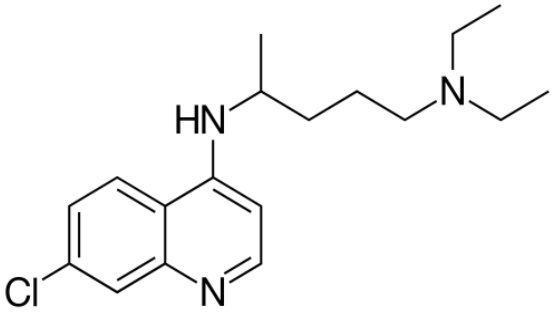
ABSTRACT

In the present research, a simple, isocratic, new rapid, economical and cost-effective reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantification of simultaneous estimation of Chloroquine and Primaquine in its bulk and pharmaceutical dosage forms. High performance liquid chromatography was one of the most sophisticated methods for the analysis of compounds. The mobile phase and p^H 3.0 phosphate buffer were optimized with contains methanol, mixed with phosphate buffer in the ratio of 70:30 % v/v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) porous silica particles were taken as stationary phase. The detection was determined by using UV spectrum at 260 nm. These solutions were scanned at a constant flow rate of 0.8 ml/min, the linearity range of Chloroquine and Primaquine were measured, that is from 100-500 µg/ml of Chloroquine and 1-5µg/ml of Primaquine and linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicates accuracy and precision of the method. The percentage recovery changes from 98-102% of Chloroquine and Primaquine, LOD and LOQ were observed within range. The validated parameters met as per the guidelines of ICH and USP. It concludes that the validated method was simple, accurate, precise and linear. The established method was suitable application in bulk and pharmaceutical dosage forms with high degree of accuracy and precision.

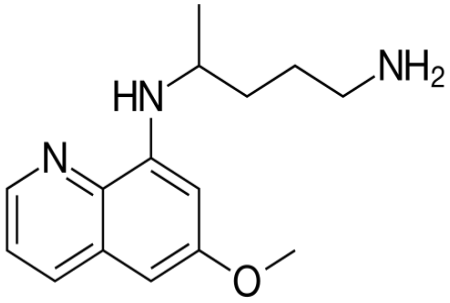
KEYWORDS: Inertsil $C_{18,}$ Chloroquine, Primaquine, RP-HPLC.

INTRODUCTION

Pharmaceutical drugs are used for the determination of their quality assurance and quality control of bulk drugs and their formulations. Qualitative analysis provides the chemical identification of the sample, and establishment of the relative amount of one or more of the species or analytes in numerical terms. A drug is a compound that has medicinal, intoxicating, performance enhancing or other effects when taken or put into a human body or the body of another animal and is not considered a food or exclusively a food. There was no single, precise definition, as there are different meanings in drug control law, government regulations, medicine, and Colloquial usage. In Chromatography, Adsorption chromatography employs high-surface area particles that adsorb the solute molecules. Usually, a polar compound such as a silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptane, octane or chloroform are utilized in adsorption chromatography. In adsorption chromatography, adsorption process is defined by competition model and solvent interaction model. In partition chromatography, the solid support was coated with a liquid stationary phase; the relative distribution of solutes between the two liquid phases determines the separation. The stationary phase can be either polar or nonpolar compound. If the stationary phase was polar and the mobile phase was nonpolar, it is known as normal phase partition chromatography. If the stationary phase was nonpolar and the mobile phase was polar, it is known as reverse-phase partition chromatography. In the present research, a simple, isocratic, new rapid, economical and cost-effective reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantification of simultaneous estimation of Chloroquine and Primaquine in its bulk and pharmaceutical dosage forms.



CHLOROQUINE



PRIMAQUINE

METHOD DEVELOPMENT

Preparation of Phosphate buffer

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The mobile phase was used as the diluant.

PREPARATION OF STANDARD & SAMPLE SOLUTION

Standard Solution Preparation

Accurately weighed and transferred 10 mg of Chloroquine and Primaquine into a 10ml & 100ml clean dry volumetric flask add 7ml of mobile phase and sonicated to dissolve completely and diluted up to the mark with the mobile phase solvent.

Sample Solution Preparation

Accurately weighed 10 tablets and crushed in mortar and pestle and transferred equivalent to 10 mg of Chloroquine and Primaquine (marketed formulation) sample into a 10ml clean dry volumetric flask add 7ml of mobile phase and sonicated to dissolve completely and diluted up to the point with the mobile phase solvent.

Procedure: Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for Chloroquine and Primaquine peaks and calculate the %Assay by using the formulae.

SYSTEM SUITABILITY

The tailing factor for the peaks due to Chloroquine and Primaquine in Standard solution could not be more than 2.0 and theoretical plates for the Chloroquine and Primaquine peaks in Standard solution could not be less than 2000.

PRECISION

Preparation of stock solution: Procedure

The standard solution was injected five times and determined the area for all five injections in HPLC, the %RSD for the area of five replicate injections were observed within the specified limits.

Acceptance Criteria

The % RSD for the area of five standard injections results could not more than 2%.

ACCURACY

Procedure

Inject the standard solutions and measure the amount found and amount added for chloroquine & primaquine and measure the individual recovery and mean recovery values.

Acceptance Criteria

The % Recovery for each level could be between 98.0 to 102.0%.

LINEARITY

Procedure

Inject each one into the chromatographic system and calculate the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and measure the correlation coefficient.

Acceptance Criteria

Correlation coefficient could be not less than 0.999.

LIMIT OF DETECTION

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank: 52µV
Signal Obtained from LOD solution: 152 µV
S/N = 152/52 = 2.9

Acceptance Criteria

S/N Ratio value could be 3 for LOD solution.

ROBUSTNESS

As part of the Robustness, deliberate change in the flow rate, mobile phase composition, temperature variation was made to calculate the impact on the method.

RESULTS AND DISCUSSION

Chromatogram for Chloroquine and Primaquine

Column : Inertsil C18 (4.6 x 250mm, 5□m)
Buffer pH : 3.0.
Mobile phase : 30% buffer 70% Methanol
Flow rate : 1.0ml per min
Wavelength : 260 nm
Temperature : ambient.
Run time : 10min.

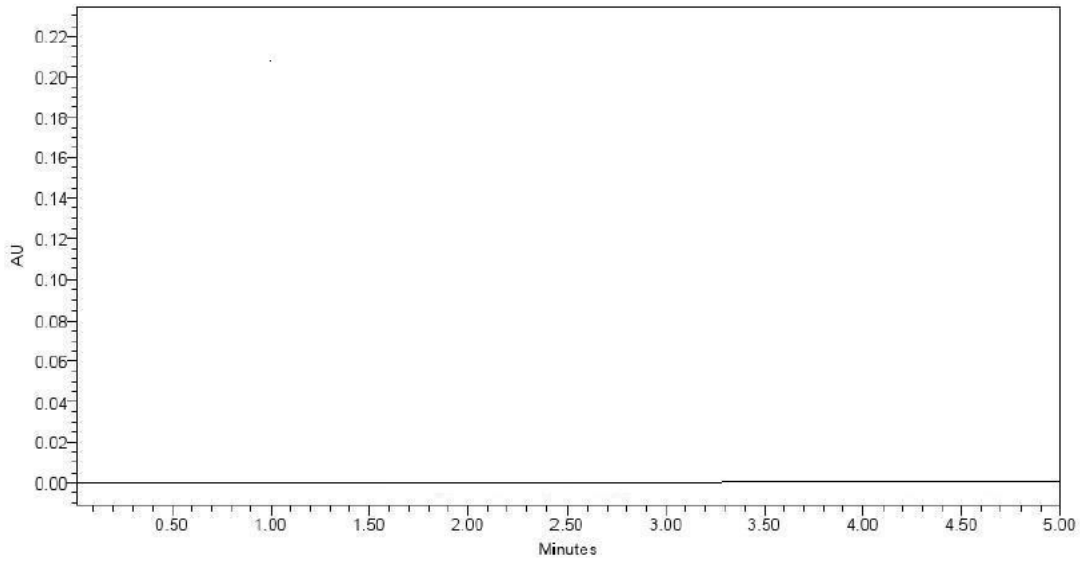


Figure 1: Chromatogram for blank.

From the above chromatogram it was found that there are no interferences.

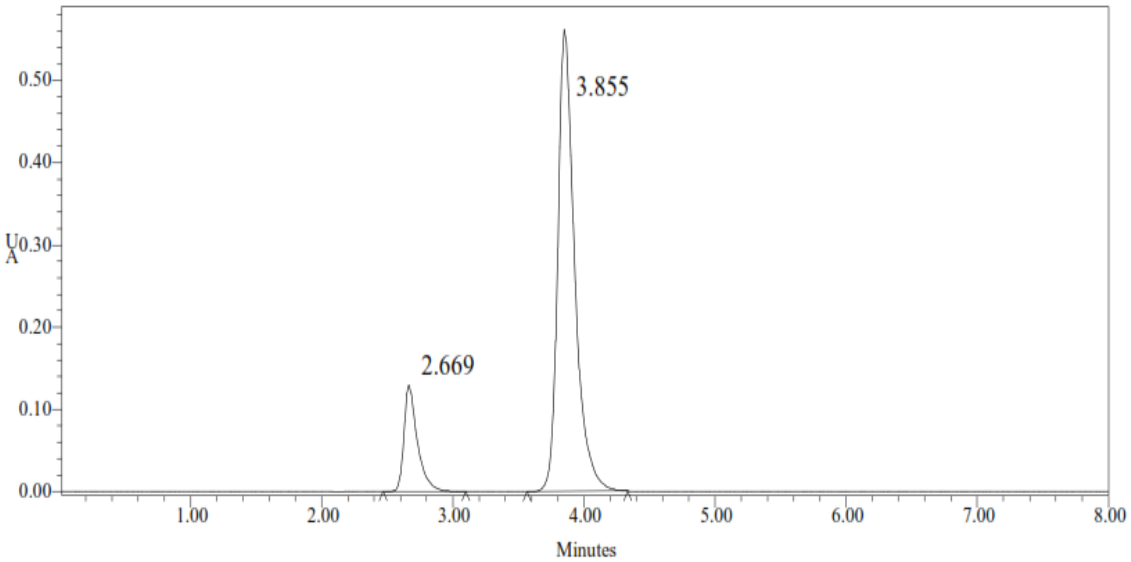


Figure 2: Chromatogram for Chloroquine and Primaquine sample Preparation.

From the above chromatogram it was found that the Chloroquine and Primaquine peaks are well separated.
Retention time of Chloroquine – 2.669min
Retention time of Primaquine - 3.855 min.

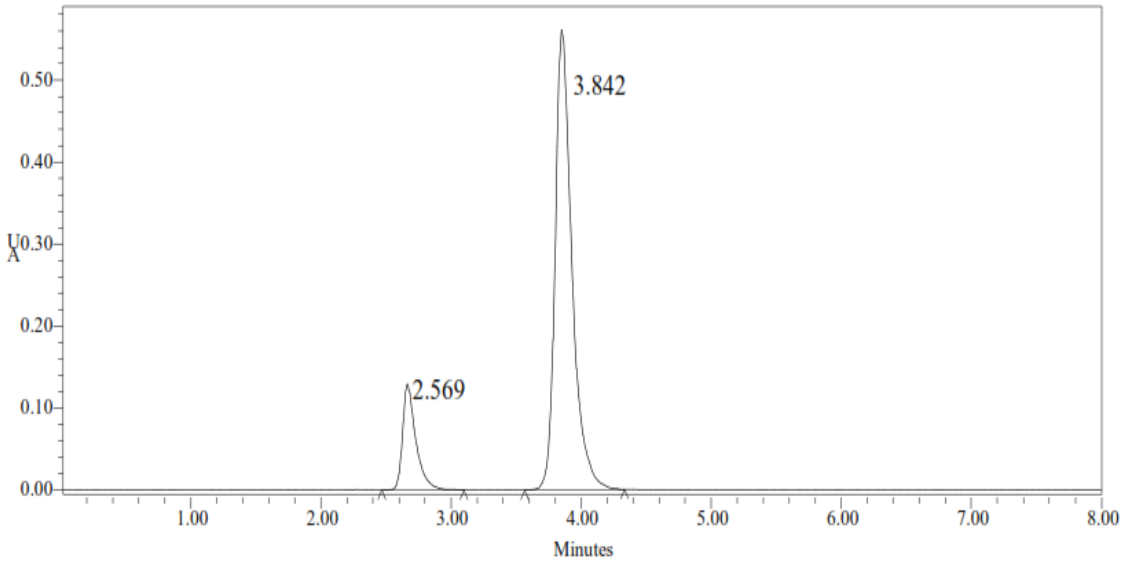


Figure 3: Chromatogram for Chloroquine and Primaquine Standard Preparation.

Retention time of Chloroquine – 2.569 min
Retention time of Primaquine - 3.842 min.

SYSTEM SUITABILITY

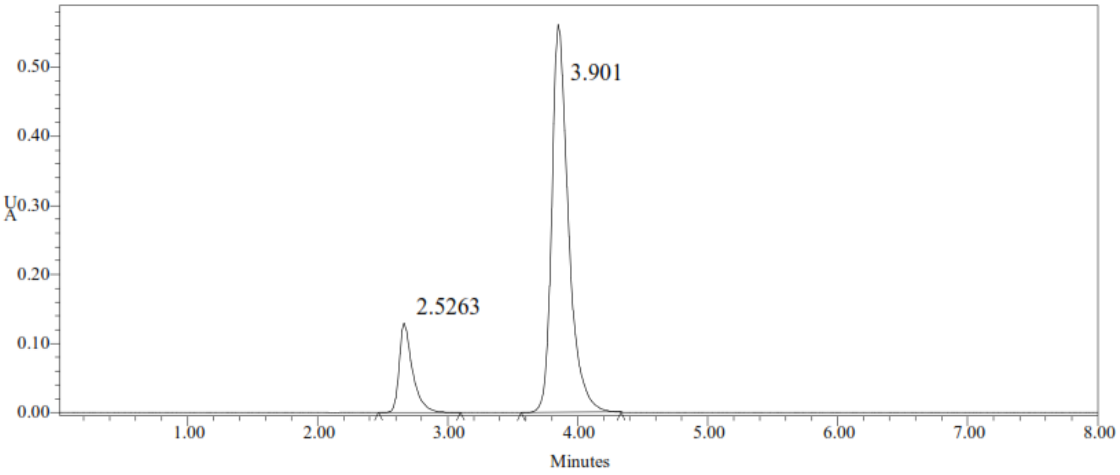


Figure 4: Chromatogram for system suitability.

VALIDATION PARAMETERS

PRECISION

Precision of the method was performed for standard solutions as described under experimental work. The corresponding chromatograms and results are shown in table 1 and 2.

Table 1: Results of method precision for Chloroquine.

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 2: Results of method precision for Primaquine.

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

ACCURACY

Different concentrations of sample solutions (50%, 100%, and 150%) are prepared and the % recovery was noted.

Table 3: Accuracy (recovery) data for Chloroquine.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 4: Accuracy (recovery) data for Primaquine.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Acceptance Criteria

- The % Recovery for each level could be between 98.0 to 102.0%.
- The percentage recovery was observed within the limit (97-103%).

The results noted for recovery at 50%, 100%, 150% are within the limits, hence method was accurate.

LINEARITY

The linearity range was found to lie from 100µg/ml to 500µg/ml of Chloroquine, 5µg/ml to 25µg/ml 0f Primaquine.

Table 5: Area of different concentration of Chloroquine.

S. No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table 6: Area of different concentration of Primaquine.

S. No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

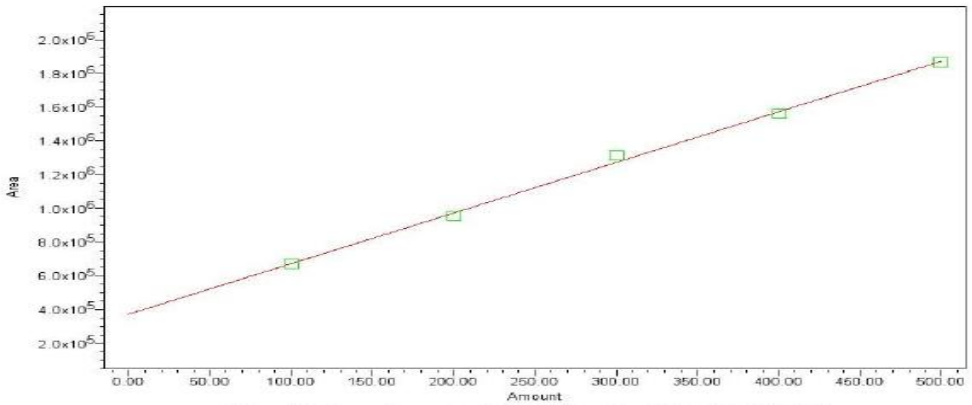


Figure 5: Calibration graph for Chloroquine at 225 nm.

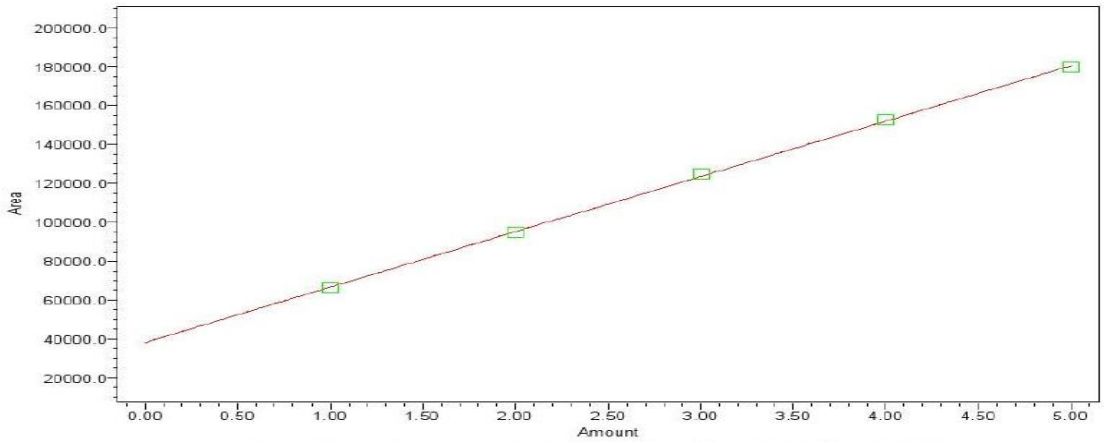


Figure 6: Calibration graph for Primaquine at 225 nm.

Table 7: Analytical performance parameters of Chloroquine and Primaquine???

Parameters	Chloroquine	Primaquine
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria

Correlation coefficient (R^2) could not be less than 0.999. The correlation coefficient obtained was 0.999 within the acceptance limit. The linearity was measured in the range of 10% to 50% of Chloroquine and 5% to 25% of Primaquine.

LIMIT OF DETECTION FOR CHLOROQUINE AND PRIMAQUINE

The sample was prepared at the lowest concentration with respect to the base line noise and measured the signal to noise ratio.

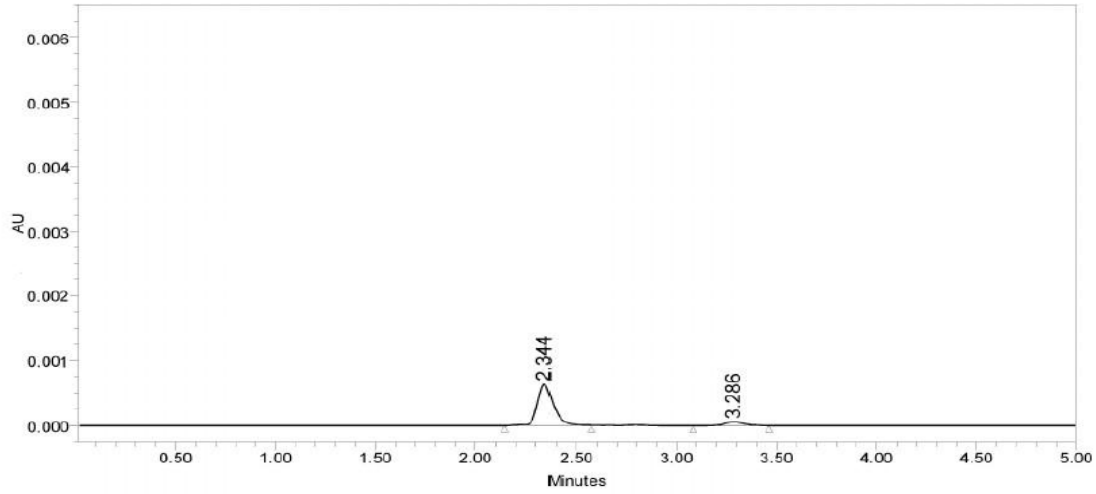


Figure 8: Chromatogram of Chloroquine & Primaquine showing LOD.

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Chloroquine	52	152	2.9
Primaquine	52	156	3

Table 10: Results of LOD.

- Signal to noise ratio shall be 3 for LOD solution.
- The result noted was within the limit.

LIMIT OF QUANTIFICATION (LOQ)

The sample was prepared at lowest concentration the with respect to the base line noise and measured the signal to noise ratio.

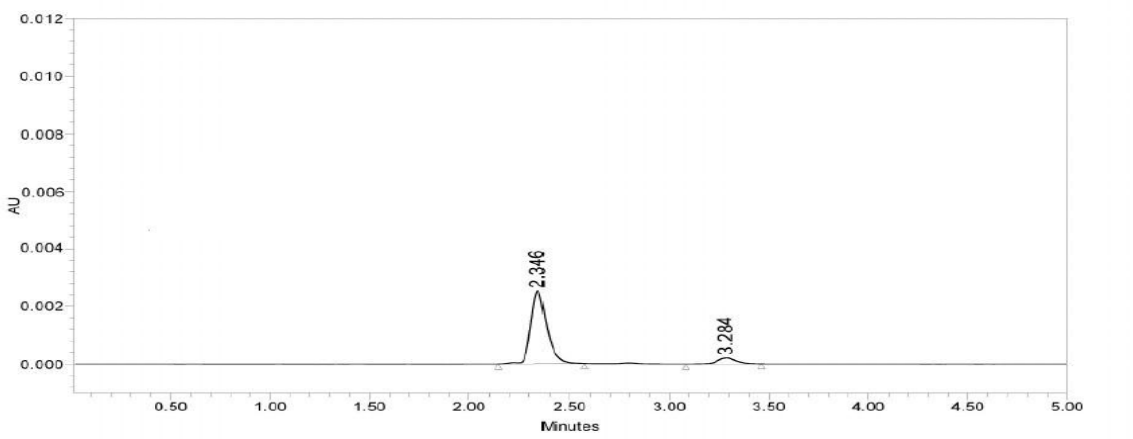


Figure 8: Chromatogram of Chloroquine & Primaquine showing LOQ.

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Chloroquine	52	522	10.03
Primaquine	52	524	10.1

Table 9 Results of LOQ.

- Signal to noise ratio shall be 10 for LOQ solution
- The result obtained is within the limit.

CONCLUSION

In the present research, a simple, isocratic, new rapid, economical and cost-effective reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantification of simultaneous estimation of Chloroquine and Primaquine in its bulk and pharmaceutical dosage forms. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) porous silica particles were taken as stationary phase. The detection was determined by using UV spectrum at 260 nm. These solutions were scanned at a constant flow rate of 0.8 ml/min, the linearity range of Chloroquine and Primaquine were measured, that is from 100-500 µg/ml of Chloroquine and 1-5µg/ml of Primaquine and linear regression coefficient was not more than 0.999. The percentage recovery changes from 98-102% of Chloroquine and Primaquine, LOD and LOQ were observed within range. It concludes that the validated method was simple, accurate, precise and linear. The established method was suitable application in bulk and Pharmaceutical dosage forms with high degree of accuracy and precision.

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