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Research Article

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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF MEFLOQUINE HYDROCHLORIDE AND ARTEMETHER IN BULK DRUG BY SIMULTANEOUS EQUATION METHOD

Amit Choudhary*, Manish Sinha, Amar Deep Ankalgi, Kamya Goyal, Arti Devi

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ABSTRACT
The purpose of the research is to establish a fast, accurate, precise, and low-cost UV-Visible spectrophotometry method for the quantitative simultaneous estimation of mefloquine hydrochloride and artemether in bulk drug. The UV-Visible method employed was a simultaneous equation method. Ethanol was used as a solvent and therefore the absorption maxima (λ max) was found to be 229 nm and 209 nm for mefloquine hydrochloride and artemether. The linearity ranges of both drugs were 1-6 µg/mL and 100 – 350 µg/mL with a regression coefficient $r^2 \geq 0.998$ respectively. The method was validated for different parameters according to International Conference on Harmonization ICH Q2B guidelines. The average recovery for mefloquine hydrochloride was found to be 100 per cent and artemether 99.3 per cent. The method was also found precise and robust with a per cent relative standard deviation of less than 2. All the parameters result obtained within the limits. Therefore, the proposed method for the accurate quantitation of mefloquine hydrochloride and artemether in the bulk drug was successfully implemented.

INTRODUCTION

Chemically mefloquine hydrochloride is (S)-[2,8-Bis(trifluoromethyl)quinolin-4-yl]-[(2R)-piperidin-2-yl]methanol;hydrochloride. It is a potent antimalarial quinoline-methanol derivative effective against chloroquine and quinine-resistant strains of *Plasmodium falciparum*. It also provided suppressive prophylaxis against mosquito-induced infections with *Plasmodium uiuax* and *P. falciparum* in human [1]. It is

* *Department of Pharmaceutical Analysis and Quality Assurance, Laureate Institute of Pharmacy, V.P.O Kathog, Jawalamukhi (H.P)176031, India*

***For Correspondence:** amit301697@gmail.com

practically very soluble in ethanol and insoluble in water. Artemether, chemically (1*R*,4*S*,5*R*,8*S*,9*R*,10*S*,12*R*,13*R*)-10-methoxy-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1. $0^{4,13}$.$0^{8,13}$]hexadecane, is an artemisinin derivative used as an antimalarial by Iron-mediated bridge cleavage [2]. Artemether is very soluble in dichloromethane & acetone and freely soluble in ethyl acetate and insoluble in water.

Several methods for determining mefloquine hydrochloride (Fig.1) and artemether (Fig.2) have been published. Some of these methods are for the determination by UV-Visible spectrophotometer [3 – 6] and RP-HPLC [7 – 9] of mefloquine hydrochloride and artemether individually [10] or in combination with other drugs. But no single method has been reported for mefloquine hydrochloride and artemether simultaneous estimation by UV and RP-HPLC. Therefore, an effort was made to develop a quick, reliable and cost-effective method for simultaneous estimation by UV-Visible spectrophotometer of both drugs.

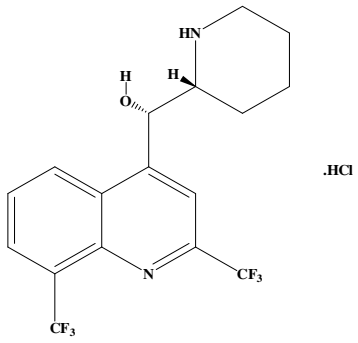

Figure 1: Chemical structure of Mefloquine hydrochloride

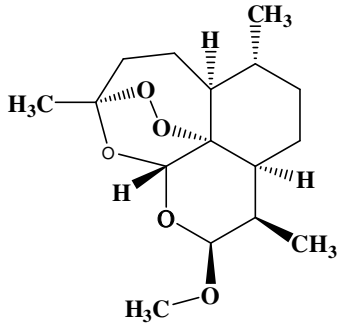

Figure 2: Chemical structure of artemether

MATERIAL AND METHODS

Chemicals and reagents

The drugs mefloquine hydrochloride and artemether were purchased from Sanjay Biological Amritsar, Punjab India. All solvents used were of an analytical standard of high quality and purchased from Shri Sai laboratory Hoshiarpur, India. Every weighing was carried out on a calibrated analytical balance. Calibrated glassware's were used throughout the work.

Instrumentation

The method was developed by using Lab India 3000$^+$ double beam spectrophotometer with a spectral bandwidth of 2 nm. UV-win software was used to acquire data. The reference and test solution absorption spectra were performed over a spectrum of 200–400 nm.

Solvent Selection

The solubility of both drugs was checked in different solvents and from solubility study, ethanol was found to be a suitable solvent. Both drugs were accurately dissolved in this solvent and showing no disturbance during recording spectra.

Preparation of Standard Stock Solutions

Mefloquine hydrochloride and artemether standard stock solutions were prepared by correctly measured 100 mg of each drug and transferred into the different volumetric flask (100 mL). Both were appropriately diluted with ethanol to get a concentration of 1000 µg/mL and were kept as stock solutions.

Selection of analytical wavelength

For the selection of wavelength 4 µg/mL concentration of mefloquine hydrochloride and 300 µg/mL of artemether was scanned in the wavelength region of 200–400 nm using ethanol as blank and the λ_{max} was found to be 229 nm for mefloquine hydrochloride and 209 nm for artemether respectively.

A spectrum of mefloquine hydrochloride and artemether

The overlay spectrum of both drugs has shown in Fig.3. The wavelengths 229 nm and 209 nm were selected for mefloquine hydrochloride and artemether because at these wavelengths both drugs showed maximum absorbance.

Selection of analytical concentration range

From the standard stock solution of mefloquine hydrochloride (100 µg/mL) and artemether (1000 µg/mL), the volume of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mL and 1, 1.5, 2, 2.5, 3, 3.5 mL was pipetted out and transferred to different 10 mL of volumetric flasks and the volume was made up to 10 mL with ethanol. These concentrations were of 1, 2, 3, 4, 5, 6 µg/mL and 100, 150, 200, 250, 300, 350 µg/mL respectively. Triplicate dilutions were prepared separately on each concentration of each drug. The

prepared mefloquine hydrochloride and artemether working solutions were scanned at 229 nm and 209 nm, respectively. The absorbance was recorded for each concentration of both drugs and calibration curves were plotted against the concentrations. The result of both drugs has shown in table 1- 5 and Fig.4-7.

The absorptivity coefficients were calculated at both wavelengths of each drug. Using equations 1 & 2 the concentration of two drugs in the mixture was calculated

$C_{MFL.HCl} = A_2\, ay_1 - A_1\, ay_2 / ax_2\, ay_1 - ax_1 ay_2$ …….. 1

$C_{ART} = A_1\, ax_2 - A_2\, ax_1 / ax_2 ay_1 - ax_1 ay_2$ …….. 2

Where, A1 and A2 are the absorbances of the mixture at 229 nm and 209 nm; ax1 and ax2, absorptivities of mefloquine hydrochloride at 229 nm and 209, respectively; ay1 and ay2 absorptivities of artemether at 229 nm and 209 nm, respectively. $C_{MFL.\ HCl}$ and C_{ART} are a concentration of mefloquine hydrochloride and artemether in the mixture. The multicomponent analysis was carried out by using a simultaneous equation method. The % assay was found to be 98.73 % for mefloquine hydrochloride & 98.93 % for artemether respectively.

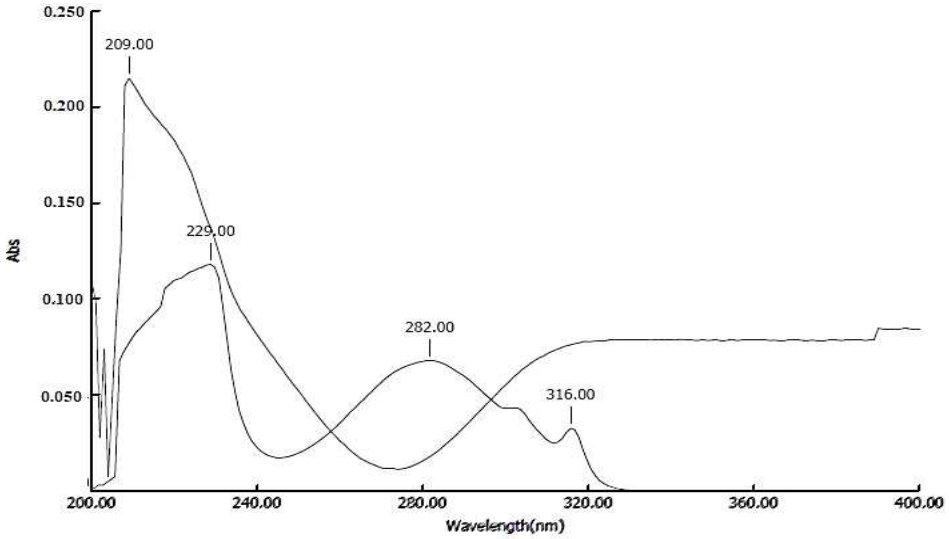

Fig 3: Overlay Spectra of mefloquine hydrochloride and artemether

Table 1: linear regression analysis of calibration curve (n=6)

Parameter	**Mefloquine hydrochloride**		**Artemether**	
Working λmax	229 nm	209 nm	209 nm	229 nm
Beer law limit	1-6 µg/mL	1-6 µg/mL	100-350 µg/mL	100-350 µg/mL
Regression equation	Y=0.0635x+0.0379	Y=0.0915x+0.0681	Y=0.008x-0.0139	Y=0.0004x-0.0171
Regression Coefficient (r^2)	0.999	0.9983	0.9995	0.9994
LOD µg/mL	0.207	0.264	9.19	9.36
LOQ µg/mL	0.628	0.802	27.8	28.3

Table 2: Results of the linearity curve for Mefloquine hydrochloride at 229 nm.

Sr. No	**Concentration (µg/mL)**	**Absorbance**
1	1	0.104
2	2	0.166
3	3	0.227
4	4	0.288
5	5	0.352
6	6	0.425

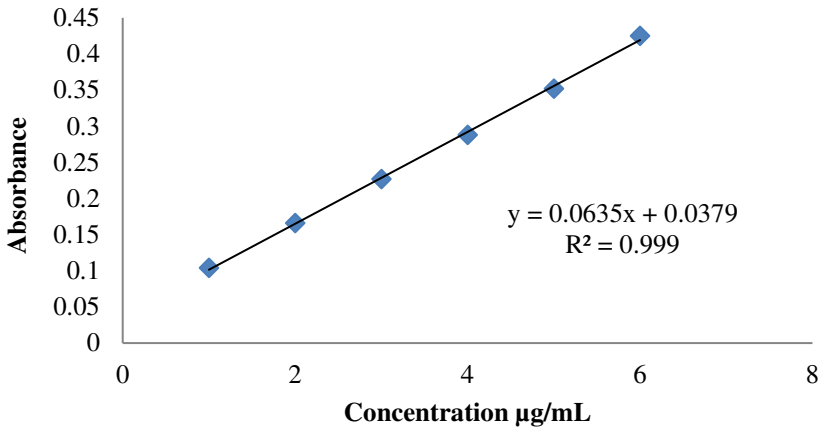

Figure 4: Calibration Curve of mefloquine hydrochloride at 229 nm

Table 3: Results of the linearity curve for Mefloquine hydrochloride at 209 nm.

Sr. No	Concentration (µg/mL)	Absorbance
1	1	0.161
2	2	0.258
3	3	0.335
4	4	0.432
5	5	0.518
6	6	0.626

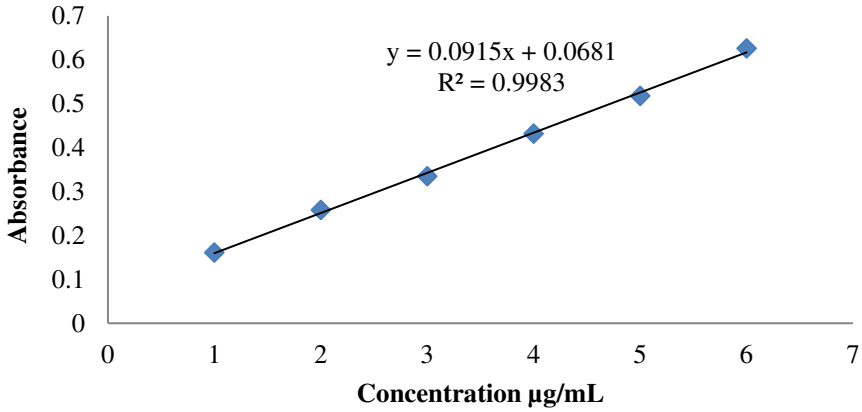

Figure 5: Calibration Curve of mefloquine hydrochloride at 209 nm

Table 4: Results of the linearity curve of Artemether at wavelength 209 nm.

Sr. No	Concentration (µg/mL)	Absorbance
1	100	0.068
2	150	0.108
3	200	0.145
4	250	0.186
5	300	0.230
6	350	0.269

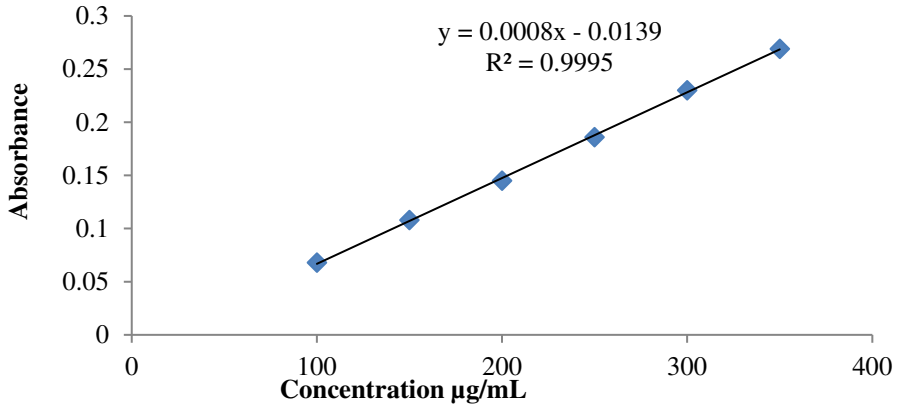

Figure 6: Calibration Curve of artemether at 209 nm

Table 5: Results of the linearity curve for Artemether at 229 nm.

Sr. No	Concentration (µg/mL)	Absorbance
1	100	0.023
2	150	0.041
3	200	0.061
4	250	0.082
5	300	0.102
6	350	0.120

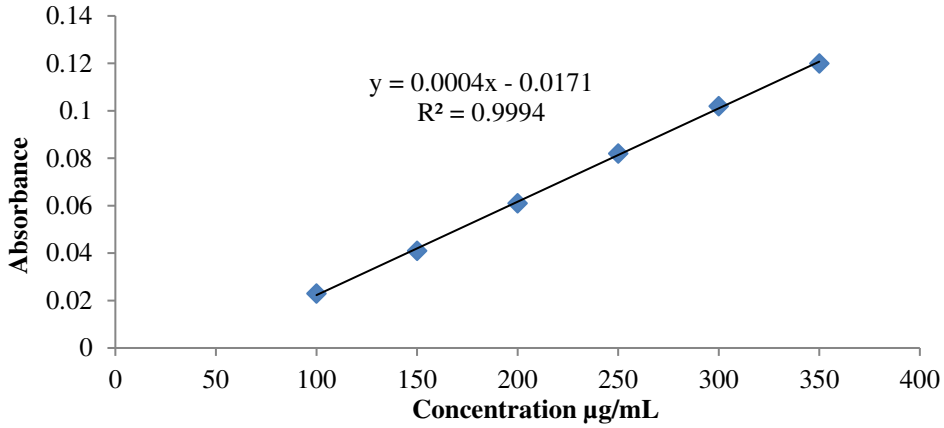

Figure 7: Calibration Curve of artemether at 229 nm

VALIDATION OF THE SPECTROPHOTOMETRIC METHOD [11]

Accuracy

Accuracy was determined by the standard addition method calculating the percentage of recovery studies at three concentration levels of 80 per cent, 100 per cent, and 120 per cent. The mean percentage recovery for mefloquine hydrochloride and artemether was found to be 100 & 99.3%. The accuracy results have been shown in table 6a & 6b

Precision

Intraday and interday precision

For intraday & Interday precision of mefloquine hydrochloride and artemether conc. 2.4, 3.0, 3.6 µg/mL and 160, 200, 240 µg/mL were prepared in three replicates from the stock solution and absorbance was taken for both. The absorbance for intraday was measured in 2 hours of interval for each set and interday result collected in different days. The method was found to be precise for intraday and interday with per cent RSD values less than 2. The results have been reported in table 7a & 7b.

Repeatability

A minimum of six determinants of 3.6 µg/mL mefloquine hydrochloride concentration and 240 µg/mL artemether concentration were prepared for repeatability, and absorbance was taken for each drug at 229 and 209 nm. The results have been reported in table 8a & 8b.

Robustness

Robustness is a measure of its capacity to remain unaffected by small, but deliberate variations in the method parameter. The robustness of the method was checked by varying the wavelength. There was a change of ± 2 nm. The concentration of 2.4 µg/mL for mefloquine hydrochloride and 160 µg/mL for artemether was prepared with their three replicates and absorbance was measured respectively. From the result data it was found that by changing the wavelength ±2 nm, there was no change in the original results The results of robustness have been reported in table 9a & 9b.

Limit of detection (LOD)

Limit of detection was calculated from the linearity curve data by excel for both drugs and calculation was made using a formula.

$$LOD = \frac{3.3 \times \sigma}{S}$$

Limit of quantitation:

For the limit of quantitation, the data was taken from the linearity curve of both drugs and the LOQ was calculated by excels. The result of both LOD and LOQ has shown in table 1.

$$LOQ = \frac{10 \times \sigma}{S}$$

Where, σ = standard deviation of the response, S = Slope

The results of LOD and LOQ have been shown in table 1.

Table 6a: Recovery Study of Mefloquine hydrochloride

Recovery Sample			**Fortified sample**			**% Recovery**
Conc.(µg/mL)	**Absorbance**	**Mean**	**Conc.(µg/mL)**	**Absorbance**	**Mean**	
2.4	0.181	0.180	2.4+3.0	0.408	0.410	100
	0.179			0.410		
	0.180			0.411		
3.0	0.230	0.229	3.0+3.0	0.459	0.459	100
	0.229			0.458		
	0.228			0.460		
3.6	0.263	0.264	3.6+3.0	0.492	0.492	99.6
	0.265			0.490		
	0.264			0.493		
Mean of % Drug Recovery						**100**

S.D. =Standard Deviation *RSD = Relative standard deviation*

Table 6b: Recovery Study of Artemether

Recovery Sample			**Fortified sample**			**% Recovery**
Conc.(µg/mL)	**Absorbance**	**Mean**	**Conc.(µg/mL)**	**Absorbance**	**Mean**	
80	0.058	0.058	80+100	0.129	0.127	100
	0.057			0.127		
	0.059			0.126		
100	0.068	0.069	100+100	0.138	0.138	100
	0.070			0.139		
	0.069			0.137		
120	0.089	0.089	120+100	0.159	0.157	98
	0.088			0.157		
	0.090			0.156		
Mean of % Drug Recovery						**99.3**

Table 7a: Intraday and Interday precision of Mefloquine hydrochloride at 229 nm and 209 nm.

Parameter	Mefloquine hydrochloride (229nm)			Mefloquine hydrochloride (209nm)		
Concentration (µg/mL)	**2.4**	**3.0**	**3.6**	**2.4**	**3.0**	**3.6**
Precision intraday %RSD	0.571	0.442	0.769	0.746	0.306	0.525
Interday-1 % RSD	0.555	0.446	0.386	0.366	0.307	0.160
Interday-2 %RSD	0.546	0.444	0.769	0.362	0.305	0.545
Interday-3 %RSD	0.847	0.442	0.584	0.740	0.612	0.539

Table 7b: Intraday and Interday precision of Artemether at 209 nm and 229 nm.

Parameter	Artemether (209nm)			Artemether (229nm)		
Concentration (µg/mL)	**160**	**200**	**240**	**160**	**200**	**240**
Precision intraday %RSD	0.793	0.672	0.562	1.18	0.984	0.841
Interday-1 % RSD	0.813	0.649	0.565	1.333	1.02	0.833
Interday-2 %RSD	0.453	0.963	0.523	1.17	0.968	0.829
Interday-3 %RSD	0.826	0.680	0.559	1.21	0.984	0.763

Table 8a: Repeatability of Mefloquine hydrochloride at 229 nm and 209 nm

Sr. No.	Absorbance (229 nm)	Sr. No.	Absorbance (209 nm)
1	0.258	1	0.375
2	0.259	2	0.377
3	0.260	3	0.372
4	0.261	4	0.371
5	0.257	5	0.370
6	0.259	6	0.372
Mean	0.259	**Mean**	0.373
S.D.	0.001	**S.D.**	0.003
% RSD	0.386	**% RSD**	0.804

Table 8b: Repeatability of Artemether at 209 nm and 229 nm

Sr. No.	Absorbance (209 nm)	Sr. No.	Absorbance (229 nm)
1	0.179	1	0.072
2	0.180	2	0.071
3	0.178	3	0.072
4	0.177	4	0.072
5	0.180	5	0.071
6	0.181	6	0.072
Mean	0.179	**Mean**	0.072
S.D.	0.001	**S.D.**	0.001
% RSD	0.559	**% RSD**	0.721

Table 9a: Robustness data of Mefloquine hydrochloride

Mefloquine hydrochloride 229± 2 nm (2.4 µg/mL)				**Mefloquine hydrochloride 209± 2 nm (2.4 µg/mL)**			
Wavelength	**227nm**	**229nm**	**231nm**	**Wavelength**	**207nm**	**209nm**	**211nm**
	0.195	0.183	0.171		0.260	0.278	0.289
Absorbance	0.193	0.182	0.172	**Absorbance**	0.262	0.276	0.291
	0.194	0.181	0.173		0.261	0.277	0.290
Mean	0.194	0.182	0.172	**Mean**	0.261	0.277	0.290
S.D.	0.001	0.001	0.001	**S.D.**	0.001	0.001	0.001
% RSD	0.515	0.549	0.581	**% RSD**	0.385	0.361	0.345

Table 9b: Robustness data of Artemether

Artemether 209±2 nm (160 µg/mL)				**Artemether 229 ±2 nm (160 µg/mL)**			
Wavelength	**207nm**	**209nm**	**211nm**	**Wavelength**	**227nm**	**229nm**	**231nm**
	0.132	0.121	0.111		0.053	0.046	0.039
Absorbance	0.133	0.123	0.113	**Absorbance**	0.054	0.047	0.038
	0.131	0.122	0.112		0.054	0.046	0.039
Mean	0.132	0.122	0.112	**Mean**	0.054	0.046	0.039
S.D.	0.001	0.001	0.001	**S.D.**	0.0006	0.0006	0.0006
% RSD	0.757	0.819	0.893	**% RSD**	1.11	1.304	1.54

CONCLUSION

In conclusion, developed UV-Visible spectrophotometer method gave the promising results of validation studies. The method's accuracy was conducted in terms of recovery analysis and mean recovery for mefloquine hydrochloride and artemether was found to be 100 & 99.3 per cent. The intraday and inter-day precision results were found to be less than 2 in terms of a per cent relative standard deviation. The proposed Vierordt's method was found to be simple, precise, robust and cost-efficient. This method can be employed in future for the determination of mefloquine hydrochloride and artemether in bulk drug.

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FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Amit Choudhary has performed all the experimental work in lab, contributed in calculation and wrote the manuscript file. Amardeep Ankalgi has contributed in calculation and also have checked the manuscript file. Manish Sinha has played a role in experimental study. He has checked all the recorded parameters during study. He has also checked the final manuscript file. Kamya Goyal have checked the final manuscript file and also contributed in writing the manuscript and final proof reading. Arti Devi have checked the final manuscript file and also contributed in writing the manuscript and final proof reading

REFERENCES

[1] Nogueira FHA, Goulart LDPL, César IDC, De Campos LMM, Pianetti GA. Development and validation of an HPLC method for mefloquine hydrochloride determination in tablet dosage form. *J. AOAC Int.*, **94**, 1089–93 (2011).

[2] Goud VM, Deepika S, Gupta A, Sreekanth M, Swamy LK. A Review on Analytical methods for the determination of Artemether in Pharmaceutical Formulation. *Asian J. Pharm. Anal.*, **10**, 112 (2020).

[3] Kalyankar TM, Kakade RB, Attar MS, Kamble AR. Simultaneous spectrophotometric estimation of artesunate and mefloquine. *J. Chem.*, **2013**, 5 (2013).

[4] Karajgi S. R. TAR and KNV. Simultaneous Determination of Artemether and Lumefantrine by Area Under Curve UV Spectrophotometric Method. *J. Pharm. Sci. Res.*, **8**, 506–11 (2016).

[5] Christian J, Shah P, Patel M, Patel K, Gandhi T. Optimizing derivatization conditions using an experimental design and simultaneous estimation of artemether and lumefantrine by ratio first order derivative spectrophotometric method. *J. Taibah Univ. Sci.*, **11**, 729–40 (2017).

[6] Arun R, Smith A. Simultaneous HPLC-UV method for the estimation of artemether and lumefantrine in tablet dosage form. *Int. J. Pharm. Biomed. Res.*, **2**, 201–5 (2011).

[7] Ahmad H, Khandelwal K, Pachauri SD, Gunjan S, Tripathi R, Dwivedi AK. HPLC analytical method for simultaneous estimation of mefloquine and clarithromycin. *J. Biomater. Tissue Eng.*, **4**, 567–72 (2014).

[8] Vinodh M, Vinayak M, Pankaj P, Rahul K, Renita M. Analytical method development and validation for simultaneous estimation of artemether and lumefantrine in pure and pharmaceutical dosage form using RP-HPLC method. *Malaysian J. Anal. Sci.*, **17**, 348–58 (2013).

[9] Habib R, Shoaib MH, Ahmed FR, Siddiqui F, Yousuf RI, Saleem T, Khan MZ. HPLC-UV method for simultaneous quantitation of artemether and lumefantrine in fixed dose combination orodispersible tablet formulation. *Pak. J. Pharm. Sci.*, **33**, 1561–7 (2020).

[10] Rao A, Murthy R. A rapid spectrophotometric method for the determination of mefloquine hydrochloride. *J. Pharm. Biomed.*, **27**, 959–65 (2002).

[11] "ICH Official web site : ICH".: <https://www.ich.org/page/quality-guidelines>, cited 30 December, 2019.