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Spectrophotometric Determination of Amodiaquine in Bulk and in Pharmaceutical Formulation

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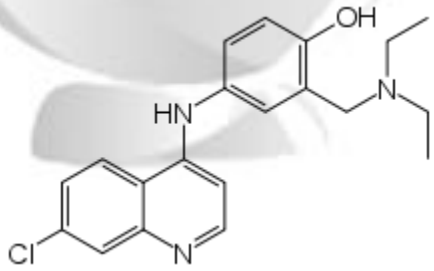
ABSTRACT

A simple, rapid, accurate and economical spectrophotometric method has been developed for estimation of amodiaquine from bulk and pharmaceutical formulation. The λ_{max} of amodiaquine in methanol and water was found to be 348 nm. The same spectrum was derivatised in to first order derivative; showed maximum amplitude of the trough at 348 nm. The drug follows linearity in the concentration range 2-12 µg/ml with correlation coefficient value 0.998. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 99.19 **% %** was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120 %. The % recovery was found to be in the range 98.54%– 99.98%. The low values of % R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than 2 indicate that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts. The above method was a rapid and cost-effective quality-control tool for routine analysis of amodiaquine in bulk and in pharmaceutical dosage form.



Keywords: Amodiaquine, spectrophotometric, quantitative determination, Double distill water.

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INTRODUCTION

Amodiaquine is chemically 4-(7-chloro-4-quinolyamino)-2-diethylaminoethyl) phenol Dihydrochloride dehydrate (Figure 1), having molecular formula $C_{20}H_{22}ClN_3O$ with molecular weight 355. It is yellow crystalline powder with melting point 208-209^{0}C and soluble in water and alcohol [1, 2]. It is acts as an anti-malerial agent. Since AMQ still has a high degree of efficacy against all chloroquine-resistant strains, there has been a recent increase in its use. However, monitoring of effectiveness and surveillance for evidence of toxicity are still being maintained. Various methods are reported for the analysis of individual drug as HPLC [3], colorimetric [4] and in combination [5-7] but no spectrophotometric method was reported estimation of drug in pharmaceutical dosage form. Accordingly, the objective of this study was to develop and validate the spectrophotometric method for the estimation of amodiaquinein bulk and pharmaceutical formulation as per ICH guidelines [8].

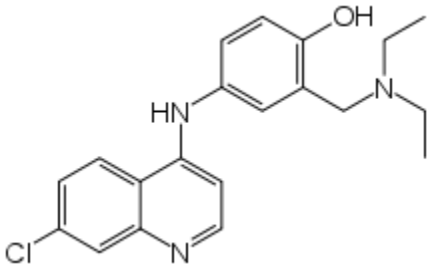

Figure 1. Chemical structure of amodiaquine

MATERIALS AND METHODS

Materials

Amodiaquine (AMQ) was supplied as a gift sample by Mepro pharmaceutical Ltd. (Gujarat). All chemicals and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

Preparation of standard stock solution

Accurately weighed 10 mg of AMQ was transferred to 100 ml volumetric flask, dissolved in 20 ml distilled water by shaking manually for 10 min. The volume was adjusted with the same up to the mark to give final strength i.e. 100 µg/ml.

Selection of wavelength for analysis of AMQ

Appropriate volume 0.2 ml of standard stock solution of AMQ was transferred into 10 ml volumetric flask, diluted to mark with distilled water to give concentration of 2µg/ml. The resulting solution was scanned in UV range (200 nm – 400 nm). In zero order spectrum AMQ showed absorbance maximum at 348 nm (Figure 2).

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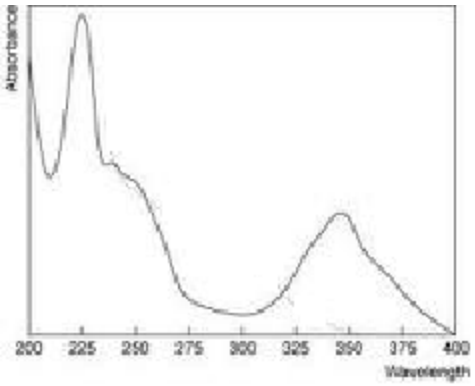

Figure 2: UV Spectrum of AMQ at 348 nm

Validation of the method

The method was validated in terms of linearity, accuracy, precision, and ruggedness.

Linearity study

Different aliquots of AMQ in range 0.2-1.2 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with distilled water to get concentrations 2, 4, 6, 8, 10 and 12 μg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200 - 400 nm. The calibration plot was constructed as concentration vs. amplitude (Figure 3).

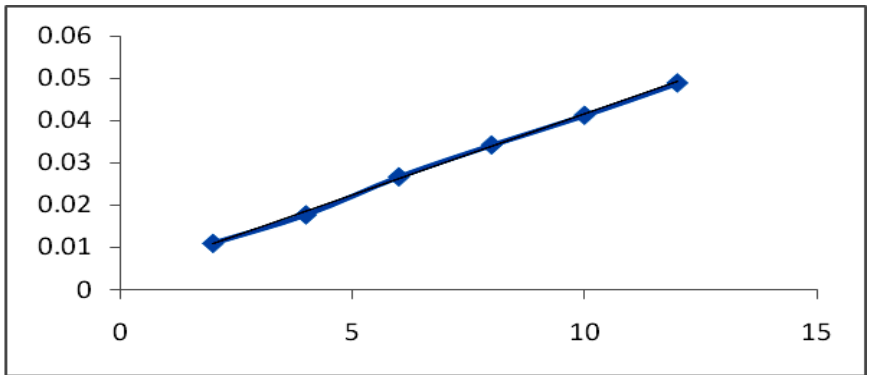

Figure 3: Calibration curve of AMQ at 348nm

Accuracy

To the preanalysed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120 %. The solutions were reanalyzed by proposed method.

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 4, 6 and 8 µg/ml of AMQ solutions for three times in the same day. Inter-day precision was determined by analyzing the 4, 6 and 8 µg/ml of AMQ solutions daily for three days over the period of week.

Sensitivity

The sensitivity of measurements of AMQ by the use of the proposed method was estimated in terms of the Limit of Quanfication (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability

Repeatability was determined by analyzing 6 µg/ml concentration of AMQ solution for six times.

Ruggedness

Ruggedness of the proposed method is determined for 6 µg/ml concentration of AMQ by analysis of aliquots from homogenous slot by two analysts using same operational and environmental conditions.

Determination of AMQ in bulk

Accurately weighed 10 mg of AMQ was transferred into 100 ml volumetric flask containing 20 ml distilled water and volume was made up to the mark using same. Appropriate volume 0.6 ml of this solution was transferred to 10 ml volumetric flask and volume was adjusted to mark using distilled water. The resulting solution was scanned on spectrophotometer in the UV range 200 - 400 nm and absorbance was measured at 348 nm. The concentrations of the drug were calculated from linear regression equations.

Application of proposed method for pharmaceutical formulation

For analysis of commercial formulation 10 ml of amodiaquine infusion was taken in 100 ml volumetric flask and the volume was made up to the mark with distilled water to give 100µg/ml concentration. From this 0.6 ml was taken and transferred to 10 ml volumetric flask and volume was made up to the mark with distilled water to give 6 µg/ml concentration. It was scanned on spectrophotometer in the UV range 200 - 400 nm. The spectrum was recorded at 348 nm. The concentrations of the drug were calculated from linear regression equation.

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RESULTS AND DISCUSSION

Method Validation

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Linearity studies

The linear regression data for the calibration curves showed good linear relationship over the concentration range 2 – 12 µg/ml for AMQ. Linear regression equation was found to be Y = 0.0038 X + 0.0033 (r^2 = 0.998). The result is expressed in Table 1.

Table 1: Linearity study of AMQ

Sl. No.	Concentration µg/ml	Absorbance* Mean ± S.D. (n=6)	% R.S.D.
1	2	0.0110 ± 0.0001	1.21
2	4	0.0178 ± 0.0002	1.63
3	6	0.0262 ± 0.0004	1.66
4	8	0.0343 ± 0.0006	1.83
5	10	0.0413 ± 0.0004	1.15
6	12	0.0489 ± 0.0006	1.37

* average of five estimations

Accuracy

The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 2 which showed that the % amount found was between 98.54%– 99.98%. with %R.S.D. >2.

Table 2: Recovery studies

Pre-analyzed sample solution (µg/ml)	Amount of drug added (µg/ml) (n=3)	Amount recovered* (µg/ml) (n=3)	% Recovery	% R.S.D.
6	0	5.91	98.54	1.38
	4.8	10.79	99.98	1.40
	6	11.8	98.68	1.44
	7.2	13.14	99.54	1.33

*average of three estimates

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation (Table 3).

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Table 3: Precision studies

Component	Concentration (µg/ml)	Intra-day precision* (n=3)		Inter-day Precision* (n=3)	
		Conc. found	% R.S.D.	Conc. found	% R.S.D.
AMQ	4	3.95	1.47	3.99	1.43
	6	5.95	0.54	5.92	0.61
	8	7.99	1.24	7.94	1.13

*average of three estimates

Sensitivity

The linearity equation was found to be Y = 0.0038 X + 0.0033. The LOQ and LOD for AMQ were found to be 0.23 µg and 0.72 µg, respectively.

Repeatability

Repeatability was determined by analyzing 6 µg/ml concentration of AMQ solution for six times and the % amount found was between 98% to 102% with % R.S.D. less than 2 (Table 4).

Table 4: Repeatability studies

Component	Amount taken (µg/ml) (n=6)	Amount found* (%)	%R.S.D.
Amodiaquine	6	99.63 ± 0.38	0.64

*average of six estimations

Ruggedness

Peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 5**.** The result showed that the % R.S.D. was less than 2%

Table 5: Ruggedness studies

Component	Amount taken (µg/ml) (n=3)	Amount Found (%) *	
		Analyst I ±S.D.	Analyst II ±S.D.
Amodiaquine	6	99.04 ± 1.3	98.90 ± 0.95

*average of six estimations

Determination of AMQ in bulk

The concentrations of the drug were calculated from linear regression equations. The % amount found was between 99.12% to 100.43% % (Table 6).

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Table 6: Analysis of AMQ in bulk

Concentration (µg/ml)	Amount found (µg)	Amount found (%)
6	5.94737	99.12
	5.97368	99.56
	5.97368	99.56
	5.71053	95.17
	5.97368	99.56
	6.02632	100.43
Mean ± S.D.	5.93 ± 0.102	98.90 ± 1.71
% R.S.D.	1.73	1.73

Application of proposed method for pharmaceutical formulation

Table 7: Analysis of AMQ in infusion

Conc. (µg/ml)	Amount found (µg)	Amount found (%)
6	5.89474	98.24
	5.97368	99.56
	5.84211	97.36
	5.97368	99.56
	5.94737	99.12
	6.07895	101.31
Mean ± S.D.	5.95 ± 0.08	99.19 ± 1.34
% R.S.D.	1.35	1.35

The spectrum was derivatised into first order derivative and amplitude of the trough was recorded at 262 nm. The concentrations of the drug were calculated from linear regression equation. The % amount found was between 98.24% to 101.31% % (Table 7).

CONCLUSION

This UV spectrophotometric technique is quite simple, accurate, precise, reproducible and sensitive. The first order UV derivative method has been developed for quantification of amodiaquine in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the plant material and formulation. It is also used in routine quality control of the raw materials as well as formulations containing this entire compound.

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