

Research Article



A Stability Indicating HPLC Method for the Determination of Amodiquine Hydrochloride

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ABSTRACT

Stability indicating HPLC method has been developed for specific determination of Amodiquine hydrochloride during degradation under acid, alkali, thermal and oxidative stress conditions. Acn: Phosphate buffer pH (5.8) with ortho phosphoric acid (80:20), was used as the mobile phase, at a flow rate of 1 ml/min. A Grace smart C18 column (5 μ), 344 mm, 2.6 mm i.d. was used as stationary phase. The retention time was approximately 2.6 min. Linearity range was 10-50 μ g/ml. The minimum detection and Quantitation limits were 0.1605 and 0.53 μ g/ml respectively. Proposed method was validated for precision, accuracy, ruggedness, robustness and specificity.

Keywords: Amodiquine, Forced degradation, HPLC, Stability, Stress.

INTRODUCTION

Amodiquine hydrochloride (4-(7-Chloro-4-quinolyamino)-2-(Di-ethyl-amino-methyl) Phenol Dihydrochloride Dihydrate), is an Antimalarial.¹ The chemical structure is shown in figure 1. Amodiquine HCL inhibits the glutathione-dependent destruction of ferriprotoporphyrin IX in the malaria parasite, resulting in the accumulation of this peptide, which is toxic for the Parasite. In general, 4-aminoquinoline derivatives appear to bind to nucleoproteins and inhibit DNA and RNA polymerase. Knowledge from the stability studies is used in the development of manufacturing process, selection of proper packaging and storage conditions, and determination of product shelf life. Literature survey reveals few analytical methods for the determination of Amodiquine in combination by HPLC², UV³ UPLC⁴ methods. The objective of this work was to develop a validated Stability indicating assay method for the determination of Amodiquine hydrochloride in presence of its degradation products generated by subjecting the drug to forced degradation conditions under acid, alkali, thermal and oxidative stress as per the guidelines⁵ and to establish the inherent stability of Amodiquine hydrochloride.

MATERIALS AND METHODS

Materials and Reagents

Amodiquine hydrochloride was supplied by Ipca Pharmaceuticals, Aurangabad as gift sample. Its identity and purity was confirmed by recording the FTIR spectra. Solvents used were HPLC grade Methanol (Merck) and water from the water treatment plant (SG) of the Institute. The reagents used were Orthophosphoric acid & hydrochloric acid, Sodium hydroxide & Hydrogen peroxide solution.

Apparatus

Dionex Liquid Chromatography, equipped with Dionex P 680 ALPG-4 quadrant pump system, ASI 100 Auto-sampler injection system, UVD 170U multi wavelength quadrant detector and Chromeleon chromatography management software was used. The column used was Gracesmart C18 (5 μ), 250 mm, 4.6 mm i.d., pH meter (Equiptronics EQ-621) and analytical balance (Citizen)

Methods⁶⁻⁸

Chromatographic Conditions

Chromatographic analysis was carried out at ambient temperature. The mobile phase consists of Acn: Phosphate (80:20 v/v) at a flow rate 1 ml/min. The detection wavelength was 344 nm. All the solvents were filtered through 0.45 μ nylon membrane filter and degassed offline by ultra-sonication. The retention time was approximately 2.6 min. Figure 1.

Preparation of Stock and Standard solutions

The stock solution (1 mg/ml) was prepared by dissolving 100 mg of Amodiquine hydrochloride in up to 100 ml HPLC grade water. It was filtered and ultra-sonicated. The standard drug solutions in the concentration range 10-50 μ g/ml was prepared by the dilution of suitable stock solution with HPLC grade water.

Preparation of calibration curve

The calibration curve was prepared in the concentration range 10-50 μ g/ml by injecting 10 μ l of each solution in triplicate and plotting the area under peak (mAU*min) against concentration (μ g/ml). The correlation coefficient and equation of the line was determined. The Chromatogram of fresh drug solution (10 μ g/ml, 10 μ l) is shown in Figure 1.



Forced Degradation Studies

The forced degradation of Amodiquine was done in each of the stress condition at a concentration of 1 mg/ml. The degradation was confirmed in each case by recording the changes in the ultraviolet spectra of each stressed sample comparing it with that of fresh drug solution.

Acidic stress

100 mg of Amodiquine hydrochloride was refluxed in 1 N Hydrochloric acid for 4 hours in a water bath at 70°C.

Alkaline stress

100 mg of Amodiquine hydrochloride was refluxed in 1 N sodium hydroxide for 4 hours in a water bath at 70°C.

Thermal stress

100 mg of Amodiquine hydrochloride was kept in solid state at 70°C for 4 hours in an oven equipped with temperature control probe.

Oxidative stress

100 mg of Amodiquine hydrochloride was dissolved in 1%, 6% Hydrogen peroxide separately and kept at room temperature for 24 hours in amber colored stoppered vials.

Recording of chromatograms of stressed samples

Suitable aliquots from each stressed sample were diluted with HPLC grade water to obtain concentration of 10 µg/ml. It was chromatographed employing the conditions mentioned above in the range 10-50 µl injecting each solution in triplicate. The chromatograms of 10 µl injection volume of 10 µg/ml of each stressed sample are shown in fig 2-6.

Method Validation⁹

Linearity and range

The linearity of the method was established by preparing a calibration curve in water in the range 10-50 µg/ml. Triplicates of each of the solution was injected and chromatograms recorded. The mean (n=3) area under peak (mAU*min) was plotted against concentration (µg/ml). The correlation coefficient and equation of line were determined.

Precision

The Interday and intraday precision were determined by calculation of the % RSD values on injection of triplicates of each concentration. The mean (n=3) area under peak (mAU*min) of each concentration was compared with that of second run on the same day (intraday) and with that on the next day (interday) and the percent relative deviation calculated.

Accuracy

Accuracy of the method was evaluated by spiking the drug at three concentration levels (80, 100 & 120 µg/ml).

The percent recovery of the added drug was calculated from the linearity plots.

Specificity

The Specificity of the method was established through the determination of the drug in the presence of its degradation products with high degree of precision. The peak homogeneity was confirmed by analyzing the ratio chromatograms at the wavelengths 344nm.

Ruggedness

Mean peak area (n=3) was measured for the 10 µg/ml solution (10 µl) analyzed by two different analysts on different days and the percent relative deviation between the runs was calculated.

Robustness

Deliberate changes in the mobile phase flow rate (+/- 0.1 units) and composition (=/- 5%) were made. Three replicates of each deviation were injected and the %RSD between the mean (n=3) area under peak (mAU*min) and that obtained under optimized chromatographic conditions were determined.

Limit of detection and limit of Quantitation

The limit of detection and limit of Quantitation were calculated based on standard deviation (σ) of responses for triplicate blank injections and the slope (S) of the calibration plot, using the formulae $LOD = 3.3\sigma/s$ and $LOQ = 10\sigma/S$ as defined by ICH.

System suitability

The system suitability parameters Theoretical plates, Asymmetry factor, Linearity and range, accuracy, precision and specificity were determined and are shown in table 1.

RESULTS AND DISCUSSION

Degradation behavior of Amodiquine hydrochloride

The amount (percent) of Amodiquine hydrochloride remaining undegraded under each stress condition was determined from the area under peak (mAU*min) of the fresh drug sample relative to that of stressed drug sample. The results are shown in table 2.

Method validation

Linearity and range

The data from the linearity curve shows that the response of the drug was strictly linear in the studied concentration range (20- 100 µg/ml). The correlation coefficient between concentration (µg/ml) and area under peak (mAU*min) was 0.999 and the equation of line was $y = 0.1041x + 0.1113$

Precision

The results of intraday and interday precision as shown in table 2 reveals that the developed method is highly



precise since the %RSD values are extremely low (limit < 2%)

Accuracy

Good recoveries were obtained in the range of 97.22 to 97.75 %, which is indicative of high accuracy.

Specificity

Specificity of the method was revealed by its ability to estimate the analyte accurately and precisely in the presence of its degradation products without interference from the latter. The asymmetry factor ranges from 1.2-1.24 while the number of theoretical plates ranges from 9000-11000. The ratio chromatograms corresponding to the drug's peak were flat in each case of forced degradation which indicates the homogeneity of these peaks.

Ruggedness

The ruggedness of the method is revealed by the low % RSD obtained between runs by two different analysts on different days as shown in table 1.

Robustness

The robustness of the developed method is revealed by the low % RSD obtained in the readings between runs done employing the optimized chromatographic conditions and that employing the deliberately altered conditions as shown in table 1.

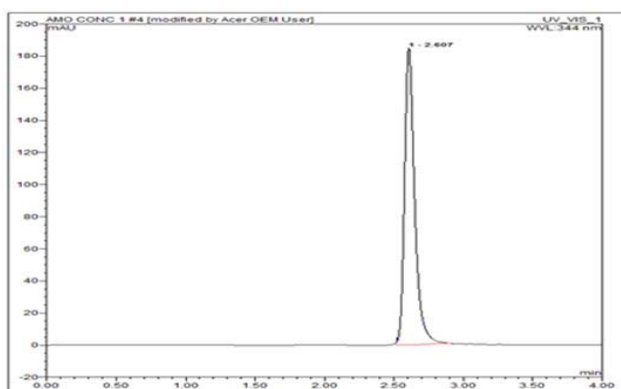


Figure 1: HPLC Chromatogram of Fresh Drug Sample at 10µL

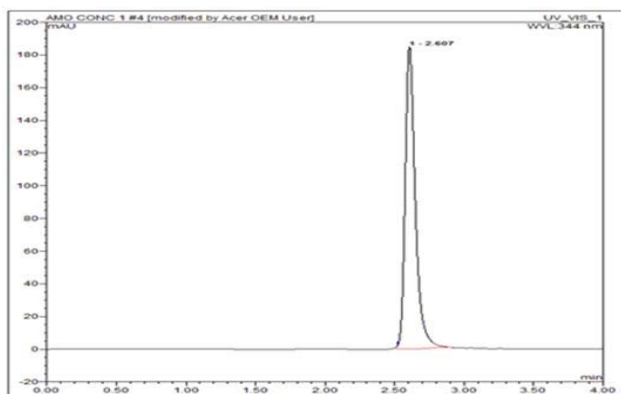


Figure 2: HPLC Chromatogram of Acid Degraded Drug Solution at 10µL

Limit of detection and limit of Quantitation

Limit of detection and limit of Quantitation were found to be µg/ml respectively 0.1605 and 0.53.

System suitability

The method meets the system suitability criteria as shown in table 1.

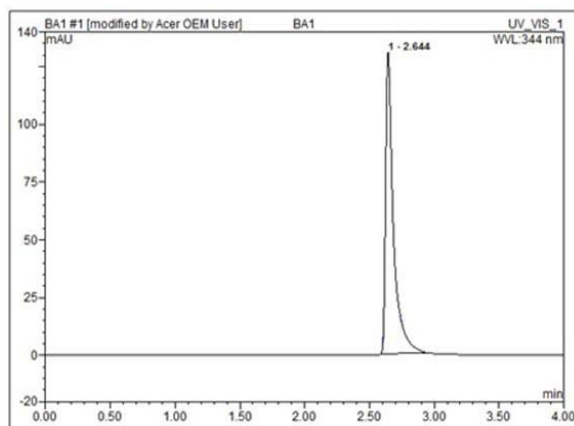


Figure 3: HPLC Chromatogram of Base Degraded Drug Solution at 10µL

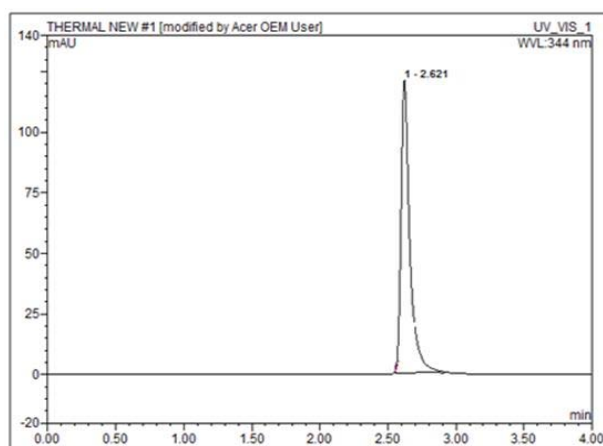


Figure 4: HPLC Chromatogram of Thermally Degraded Drug Solution at 10µL

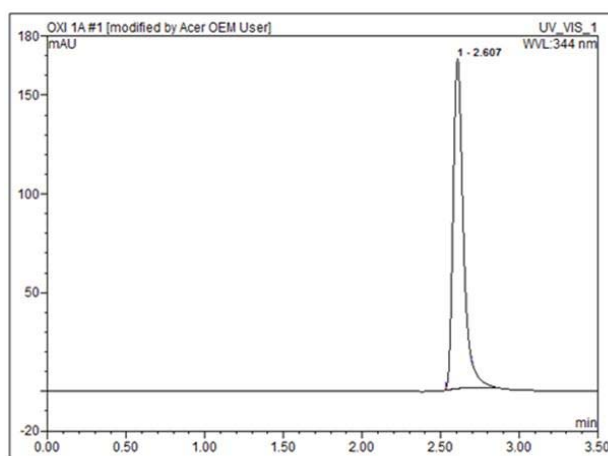


Figure 5: HPLC Chromatogram of 1% H₂O₂ at 10µL

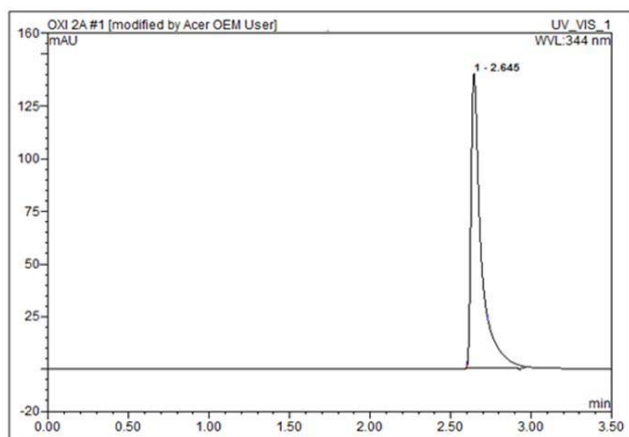


Figure 6: HPLC Chromatogram of 6% H₂O₂ at 10µL

Table 1: System Suitability Parameters

Parameters	Value
Linearity range (µg/ml)	10-50
Regression equation (y=mx+c)	Y=0.1041x+0.1113
LOD(µg/ml)	0.1605
LOQ(µg/ml)	0.53
Recovery (%RSD)	0.304
Intra-day (n=3) precision (%rsd)	0.580
Interday(n=3) precision (%rsd)	0.307
Analyst 1(n=3)ruggedness(%rsd)	0.966
Analyst 2(n=3)ruggedness (%rsd)	0.99
Sensitivity	Sensitive
Robustness	Robust

Table 2: % Drug Degraded Under Each Stress Condition

Condition	% Drugs Remaining	% Drug Degraded
Normal	100	0
Acid degraded	88.75	11.25
Base degraded	76.25	23.75
Thermally degraded	68.75	31.25
Oxidatively degraded(1%h ₂ o ₂)	99.37	0.63
Oxidatively degraded(3%h ₂ o ₂)	81.25	18.75

CONCLUSION

A validated stability indicating assay method has been developed for the determination of Amodiaquine hydrochloride in bulk and in dosage form. A new mobile phase was found during the method development process. The results show that the developed method was accurate, precise, simple, economic, fast and specific. Amodiaquine is most prone to degradation under thermal

stress, followed in order by the stress alkali, oxidative (3%), acid, oxidative (1%). The method can be considered for routine quality control and stability studies on Amodiaquine hydrochloride.

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