Research Article

UV-Spectrophotometric Method for the Estimation of Atovaquone in Bulk and Pharmaceutical Dosage form Using Hydrotropic Solubilization Technique

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ABSTRACT

Effective and advantageous Hydrotropic Solubilization technique has been developed for the estimation of Atovaquone in bulk and pharmaceutical formulation. Hydrotropic Solubilization technique is one of the aqueous solubility enhancing methods employed for the poorly water soluble drugs and found to be simple, precise and cost effective. Solvents like Piperazine, Urea, Sodium Salicylate, Sodium benzoate etc are the commonly used as hydrotropic solvents in different concentrations. In this context, solubility of Atovaquone is increased by using 1M piperazine as a hydrotropic agent. Atovaquone showed the maximum absorbance at 274 nm in method A and calculation of Area Under Curve (AUC) was done in the wavelength range of 264-284 nm in method B. The developed methods were found to be linear in the range of 4-20 µg/ml with correlation coefficients (R^2) of 0.999, 0.999 respectively. The mean percent label claim of tablets of Atovaquone in formulation estimated by the proposed methods was found to be 98.75%. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical parameters were found to be good accordance with the prescribed values. As hydrotropic agent was used in the proposed methods, these methods were eco-friendly and it can be used in routine quantitative analysis of drug in bulk and pharmaceutical dosage form.

Keywords: Atovaquone,AUC, Hydrotropic Solubilization technique, Piperazine.

INTRODUCTION

Atovaquone chemicallyis Trans-2-[4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1, 4-naphthalenedione[1].Itis white crystalline solid that is highly lipophilic and freely soluble in methanol, chloroform, DMSO and less soluble in water and ethanol[2]. It has a molecular weight of 267.36 and the molecular formula $C_{22}H_{19}ClO_3$.It is used as antimalarial drug. The drug acts by selectively affecting mitochondrial electron transport and parallel processes such as ATP and pyrimidine biosynthesis[3]. The structure of Atovaquone is shown in the figure1.Literature survey revealed that very few methods have been reported for the estimation of Atovaquonewhich include derivative UV spectroscopic(4-6) , RP-HPLC(7-10) and UPLC(11)methods either individually or in combination with other drugs. Hence the aim of the present work is to develop and validate a new simple, economical, selective, accurate, precise spectrophotometric method for the determination of Atovaquone by using hydrotropic solubilization technique in bulk and pharmaceutical dosage form and validate as per ICH guidelines.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Atovaquone (99.75%) was obtained as gift sample from spectrum laboratories, Hyderabad, India. Pharmaceutical tablet formulation of Laveran tablet containing 250mg of Atovaquone was purchased from local market. 1M Piperazine and double distilled water were used as solvents for the present study.

Instrumentation

Shimadzu UV -1800 double beam spectrophotometer with 1cm path length supported by Shimadzu UV-probe software,version 2.21 was used for spectral

measurements with 10mm matched quartz cells. Shimadzu balance (BL-220H) was used for weighing.

Selection of solvent

1M piperazine solution was used as a solvent for developing spectral characterstics of a drug. The selection was made after assessing the solubility in different hydropropic solvents like sodium acetate, sodium benzoate, urea, sodium chloride, citric acid.

Preparation of reagent solution

1M piperazine solution was prepared by weighing 8.614 gm of piperazine pure chemical and dissolved in 10 ml of double distilled water and the volume was made upto the mark with double distilled water in 100 ml volumetric flask.

Preparation of standard stock solutions

The standard solution of Atovaquone was prepared by dissolving accurately weighed 10 mg of drug in 1ml of 1M Piperazine to enhance solubility of the poorly aqueous soluble drug and then the volume was made up to 10 ml with double distilled water to obtain a final concentration of 1mg/mL.

Preparation of working standard solutions

From the above standard stock solutions, 1mL of was pipetted out into a 10mL cleaned and dried volumetric flask and the volume was made up to the mark with double distilled water to get a concentration of 100µg/mL (working standard solution).From this solution a series of aliquots were prepared for further method development.

Method A

Absorption maxima method

For the selection of analytical wavelength 10µg/ml solution of Atovaquone was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectrum λ_{max} of Atovaquone 274 nm was selected for the analysis. The calibration curve was prepared in concentration range of 4-20 µg/ml at 274 nm. The calibration curve for Atovaquone was plotted against concentration v/s absorbance and regression equation was calculated. The absorption spectrum and calibration curve of Atovaquone was shown in the figures 2& 3 respectively.

Method B

Area under curve method

The AUC (area under curve) method is one of the UV methods for the determination of drugs. This method of AUC is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. The AUC spectrum and calibration curve constructed by plotting concentration on X-axis versus AUC on Y-axis was shown in the figures 4& 5 respectively.

Estimation of Atovaquone in tablet formulation

For the estimation of Atovaquone in the commercial formulation, 20 tablets of Laveran, each containing 250 mg of Atovaquone were weighed and average weight calculated. Triturate the tablets, for the analysis of drug ,quantity of powder equivalent to 10 mg of Atovaquone was transferred to10 ml volumetric flask and dissolved in 1ml of 1M piperazine, and final volume was made up to the mark with double distilled water. It was filtered through whatmann filter paper no.41 to obtain a stock solution of 1000 µg /ml of Atovaquone. Further dilutions of the stock solution were made in double distilled water to get required concentration. In method A the concentration of Atovaquone was determined by measuring absorbance of sample solution at 274nm and in method B the concentration of Atovaquone was determined by measuring absorbance of sample solution in wavelength range of 264-284 nm. Results of the marketed formulation analysis was shown in the table 1.

Method validation

The method was validated according to ICH guidelines in terms of accuracy, linearity and precision.

Linearity

A 5-point (4.0–20.0 µg/ mL) calibration curve of Atovaquone was prepared on 3 different days. The results obtained were used to calculate the equation of the line by using linear regression by the least-squares regression analysis method. The regression equation, slope and correlation coefficient of the drug was shown in the table 2.

Accuracy

This parameter was evaluated by the percent recovery studies at concentration levels of 50, 100 and 150% which consisted of adding known amounts of Atovaquone reference material to a pre quantified sample solution. The recovery was verified by estimation of the drug in triplicate preparations at each specified concentration level. The spectrums were recorded in the UV range and then analyzed. The results are reported in terms of % recovery to state whether the method is accurate and the results were shown in the table 3.

Precision

Precision is the level of repeatability of results as reported between samples analyzed on the same day (intra-day) and samples run on 3 different days (inter-day). To check the intra-day and inter-day variation of the method, solutions containing 8,12,16 µg/ml of Atovaquone were subjected to the proposed spectrophotometric methods of analysis and the recoveries obtained were noted. The precision of proposed method i.e. the intra and inter-day variations in the absorbance of the drug solutions was calculated in terms of % RSD and the results were shown in the table 4.

LOD

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. The detection limit is usually expressed as the concentration of analyte.

The standard deviation and response of the slope-

LOD=3.3 * standard deviation (σ)/ s

LOQ

The quantitation limit of an analytical procedure is the lowest amount of an analyte of a simple which can be quantitatively determined with suitable precision and accuracy.

The standard deviation and response of the slope-

LOQ=10* standard deviation (σ)/ s

The corresponding LOD and LOQ values were shown in the table 5.

RESULTS AND DISCUSSION

The methods discussed in the present work provided a convenient and accurate way for the analysis of Atovaquone in bulk and pharmaceutical dosage forms. The absorbance maxima of Atovaquone was found to be 274nm for the method A and for method B the area under curve in the range of 264-284nm was selected for the analysis as shown in the figures 2 & 4 respectively. Linearity for both the methods was observed in the concentration range of 4-20 µg/mL as shown in the table 2. The calibration curves for both the methods were shown in figures 3 & 5 respectively.The assay for the two methods was found to be within the range of 98-102% as shown in the table 1. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. In both the intra-day and inter-day precision study for two methods the %RSD was found to be less than 2.0 indicating the good precision of the method as shown in the table 4. The validation of proposed methods was further confirmed by recovery studies, the %recovery values vary from 98- 102% as shown in the table 3. The LOD & LOQ values were shown in the table 5. Based on results obtained, it was found that the proposed methods were found to be accurate, precise and reproducible and can be employed for routine quality control analysis of Atovaquone in tablet dosage forms.

CONCLUSION

The proposed methods were found to be simple, sensitive, accurate and precise and showed no interference from the common additives and excipients. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. The proposed methods were also more economical because, here we used 1M Piperazine and double distilled water for the standard stock solution and further dilutions made with double distilled water. Hence the proposed methods can be routinely used for the estimation of Atovaquone in bulk and pharmaceutical dosage forms.

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Table 1: Results of marketed formulation analysis

Method	Label claim(mg)	Test conc(µg/ml)	Amount found (µg/ml)	%Assay
A	250mg	10	9.9	99%
B	250mg	10	9.94	99.4%

Table 2: Linearity studies of the proposed methods

S.No.	Parameter	Method A	Method B
1	Linearity(µg/ml)	4-20	4-20
2	Linearity equation	y=0.0483x+0.0077	y=0.9005x+0.1317
3	Slope	0.0483	0.9005
4	Correlation coefficient	0.999	0.999

Table 3: Accuracy studies of the proposed methods

Method	Level of recovery	Pre analyzed conc.(µg/ml)	Amount added(µg/ml)	Amount found(µg/ml)	%Recovery
A	50%	8	4	11.84	98.66%
	100%	8	8	15.80	98.77%
	150%	8	12	19.90	99.50%
B	50%	8	4	11.80	98.33%
	100%	8	8	15.92	99.50%
	150%	8	12	19.95	99.75%

Table 4: Precision studies of proposed methods

	Intra day precision			Inter day precision		
Method	Concentration (µg/ml)	Mean ±SD	%RSD	Concentration (µg/ml)	Mean ±SD	%RSD
A	8	0.390±0.0025	0.654	8	0.391±0.0027	1.128
	12	0.591±0.0003	0.805	12	0.592±0.0017	1.016
	16	0.782±0.0008	1.102	16	0.780±0.0028	1.102
B	8	7.332±0.0035	0.474	8	7.310±0.0034	1.243
	12	10.763±0.006	0.730	12	10.685±0.0029	1.104
	16	14.129±0.002	0.857	16	14.268±0.0051	0.975

Table 5: LOD &LOQ studies of the proposed methods

Parameter	Method A	Method B
LOD	0.190 µg/ml	0.01 µg/ml
LOQ	0.57 µg/ml	0.03 µg/ml

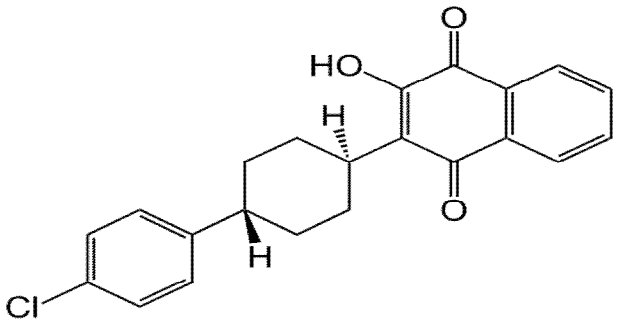

Fig. 1: Structure of Atovaquone

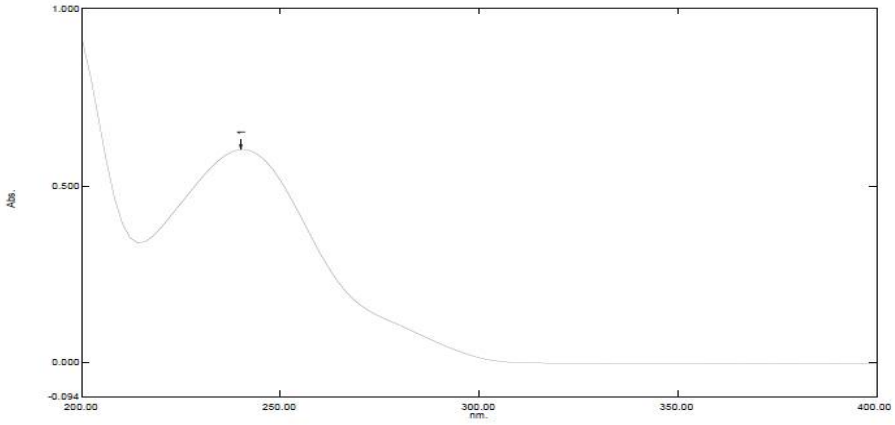

Fig. 2: Absorption maxima spectrum of Atovaquone

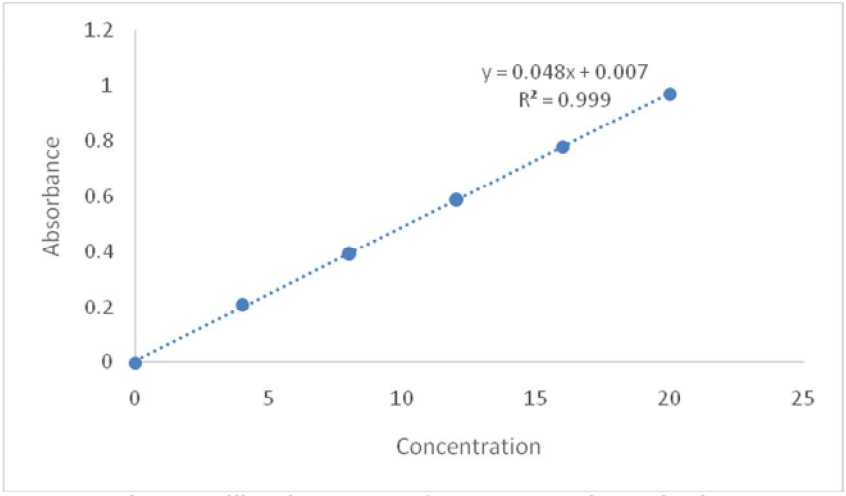

Fig. 3: Calibration curve of Atovaquone in Method A

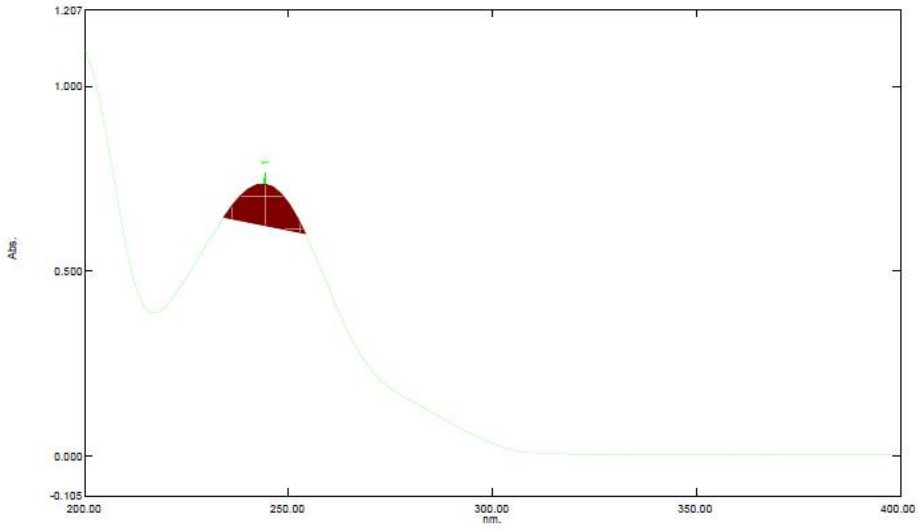

Fig. 4: AUC spectrum of Atovaquone

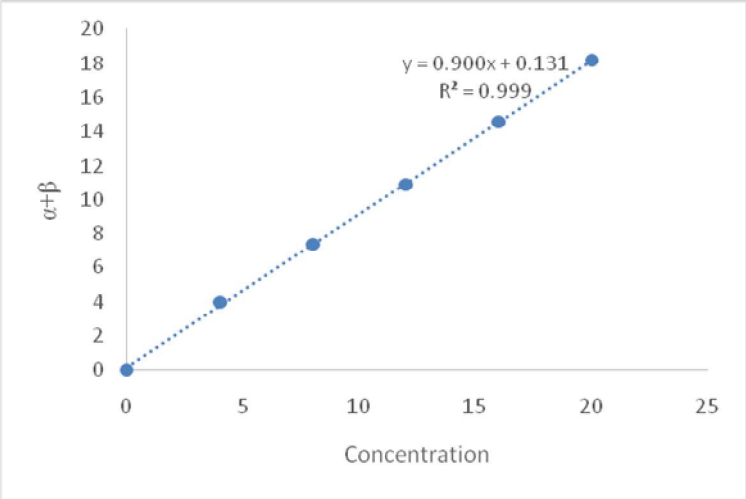

Fig. 5: Calibration curve of Atovaquone in Method B

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