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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF ATOVAQUONE IN PHARMACEUTICAL DOSAGE FORM

Bandaru Aashritha, Ashok Gorja, Durga Pani Kumar Anumolu, Syed Sara Afreen, Hirijana Upendra and Eppanoori Shivani*

Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad - 500090, Telangana, India.

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***Corresponding Author**
Eppanoori Shivani
Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad - 500090, Telangana, India.

ABSTRACT

A simple, precise, and accurate UV spectrophotometric method was developed and validated for estimating Atovaquone in marketed formulations. Methanol was used as the solvent, and the maximum absorbance was observed at 276 nm. The method showed excellent linearity in the range of 2–10 µg/mL with a regression equation of y = 0.0981x + 0.0126 and a correlation coefficient of 0.9998. The molar extinction coefficient was 39,986 $L \cdot mol^{-1} \cdot cm^{-1}$, and Sandell's sensitivity was 0.009 µg/cm². Precision was demonstrated by %RSD values of 0.82 (intra-day) and 0.64 (inter-day). Accuracy ranged from 100.56% to 100.98%. The LOD and LOQ were 0.05 µg/mL and 0.16 µg/mL, respectively. The method was specific, reliable, economical, and compliant with ICH guidelines, making it suitable for routine analysis.

KEYWORDS: UV spectrophotometry, Atovaquone, Linearity, Precision, Accuracy, LOD, LOQ, ICH guidelines, Analysis.

INTRODUCTION

Spectroscopy is the study of electromagnetic radiation absorbed or emitted by atoms or molecules as they transition between energy states. Spectrophotometry, a type of spectroscopy, measures absorption in the 190–780 nm range, covering UV and visible light. Absorption occurs due to excitation of bonding or non-bonding electrons. It is used for both

qualitative and quantitative analysis, where the absorption spectrum reveals structural information and the intensity of absorption is proportional to the substance's concentration.

The modern pharmaceutical analytical instrumentation will include

1. UV spectrophotometer
2. Infra-red spectroscopy
3. Differential thermal spectroscopy
4. X-ray spectroscopy
5. Mass spectroscopy
6. NMR
7. Atomic absorption spectroscopy
8. Liquid chromatography
9. Gas chromatography
10. Ion exchange chromatography.

Physico-chemical methods study physical changes from chemical reactions and include optical techniques (e.g., refractometry, spectrophotometry, fluorescence) and chromatographic methods (e.g., TLC, HPLC, GLC). Advanced tools like NMR, mass spectroscopy, and their combinations with chromatography are widely used. Chemical methods include gravimetric and volumetric analysis based on acid-base, redox, precipitation, and complexation reactions, including non-aqueous titrations and complexometry in pharmaceutical analysis.

Types of spectroscopies

Spectroscopy can be conveniently divided into following types based on (6-8)

1. Whether the study is made at atomic or molecular level.

a. Atomic spectroscopy

b. Molecular spectroscopy

2. Whether the study is based upon absorption or emission of EMR.

a. Absorption spectroscopy

b. Emission spectroscopy

3. Whether the study is at electronic or magnetic levels.

a. Electronic spectroscopy

b. Magnetic spectroscopy

Drug Profile

ATOVAQUONE (25)

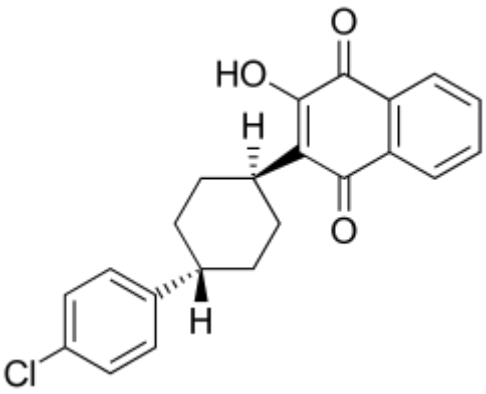

Figure 1: Chemical structure of Atovaquone.

IUPAC Name: Trans-2-[4-(4-Chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthalenedione

Molecular Formula: $C_{22}H_{19}ClO_3$

Synonym: Mepron

Molecular Weight: 366.84 g/mol

Description: Atovaquone is a chemical compound that belongs to the class of naphthoquinones. Atovaquone is a hydroxy-1,4-naphthoquinone, an analog of both ubiquinone and lawsone.

Mechanism of Action: Atovaquone can act by selectively affecting mitochondrial electron transport and parallel processes such as ATP and pyrimidine biosynthesis in atovaquone-responsive parasites. Cytochrome bc1 complex (complex III) seems to serve as a highly discriminating molecular target for atovaquone in Plasmodia.

Uses: It is an antimicrobial medication for the prevention and treatment of *Pneumocystis jirovecii* pneumonia (PCP)

Validation parameters

According to ICH guidelines, method validation confirms that an analytical procedure is suitable for its intended purpose. It involves documented evidence ensuring consistent,

accurate results that meet predefined specifications. Validation supports the identity, quality, purity, and potency of drug substances and products.

The analytical method validation parameters include

(a) Accuracy

(b) Precision

(c) Specificity

(d) Linearity

(e) Range

(f) Limit of detection

(g) Limit of quantitation

Specificity

The ability of analytical method accurately to measure the analyte response in a sample in the presence of other potential components like excipients, degradants, impurities etc this parameter is measured for identity tests, for content or potency tests, and for purity tests to ensure that the assay provides an accurate statement of the identity, potency or purity of a product. Selectivity (specificity), like accuracy, is expressed as the bias or the % error between the measured and known value.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Reproducibility expresses the precision between laboratories.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Limit of quantitation

It is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOD and LOQ are determined based on visual evaluation or based on signal to noise ratio or based on standard deviation of blank or based on calibration curve.

LOD = 3.3 σ/s σ = the standard deviation of the response

LOQ = 10 σ/s s = the slope of the calibration curve

MATERIALS AND METHODS

Method Development

Solvent selection

At the start of the method development for this drug, different solvents were tested such as Water, Methanol, 0.1N NaOH, 0.1N HCl, and acetonitrile. To select a suitable solvent for the

determination of Atovaquone, various solvents were selected for the solubility studies and it was found that Atovaquone was freely soluble in Methanol. In the present investigation, Methanol was used for all the dilutions due to greater solubility and reproducible readings of maximum absorbance.

Selection of wavelength

The absorbance of the solution containing Atovaquone was determined by scanning in the wavelength range of 200-400nm. λ_{max} of the solution was found to be 276 nm.

Preparation of stock solution (1000µg/ml)

Accurately weigh and transfer 100 mg of Atovaquone working standard into a 100 ml volumetric flask and add methanol to dissolve it completely and make volume up to the mark with methanol.

Standard solution preparation: (10µg/ml)

Pipette out 1 ml from the above stock solution into a 100 ml volumetric flask, and dilute up to the mark with methanol, mix well.

Sample solution preparation

Average weight of twenty tablets of Atovaquone (AtovaQuin) was calculated, an amount equivalent to 100 mg of Atovaquone was weighed and dissolved in 100 ml of methanol. Pipette out 1 ml from the above solution into a 100 ml volumetric flask, and dilute up to the mark with methanol.

PROCEDURE: Place the standard solution and sample solution into the UV-Visible Spectrophotometric system and measure the absorbance of Atovaquone and calculate the % Assay by using the formulae.

SELECTION OF ABSORPTION MAXIMUM

The absorption maximum of 10 µg/ml standard was found to be 276 nm.

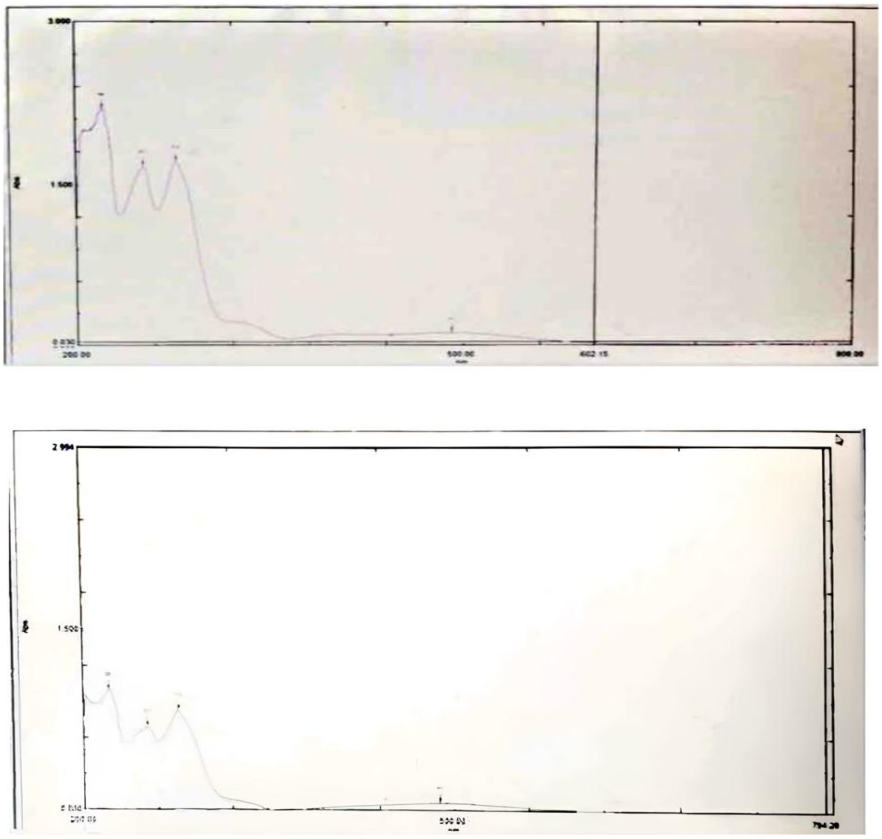

Figure 4: UV spectrum of Atovaquone.

RESULTS

1. Linearity of test method

A series of Standard solutions were prepared and placed in the UV-Visible Spectrophotometric system. A graph was plotted to "concentration" versus "absorbance" in linearity section.

Table 1: Results of calibration curve.

S. NO	CONCENTRATION (µg/ml)	ABSORBANCE
1	2	0.218
2	4	0.412
3	6	0.603
4	8	0.798
5	10	0.987
Regression coefficient (r^2)		0.9995
Correlation coefficient (r)		0.9998

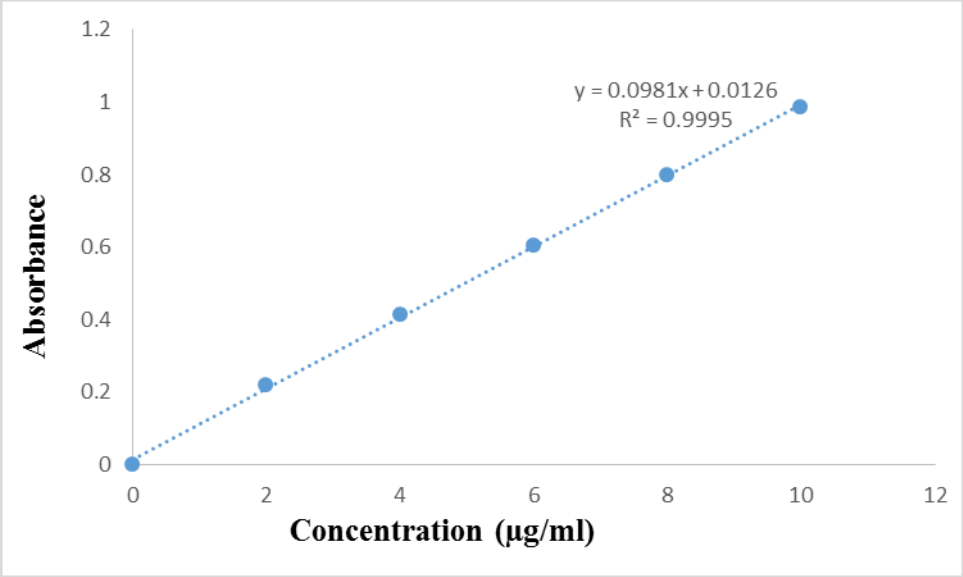

Figure 2: Linearity plot of Atovaquone.

Acceptance criteria

The correlation coefficient should be 0.999.

2. Precision

a. Repeatability (Intra-day precision)

Intra-day precision of the test method was determined by using six samples prepared by spiking Atovaquone raw material in the same day over a short interval of time. The precision of test procedure was evaluated for 100 mg by performing the assay as per the test method.

Table 2: Intra-day precision results.

Sample Name	Sample Absorbance	% Assay
1	0.262	100.57
2	0.261	100.18
3	0.258	99.03
4	0.263	100.95
5	0.259	99.42
6	0.258	99.03
Average	0.260	99.86
% RSD	0.82	0.82

b. Intermediate Precision (Inter-day precision)

Inter-day precision of the test method was determined by using six samples prepared by spiking Atovaquone raw material between days (in next three days). The precision of test procedure was evaluated for Atovaquone 100 mg by performing the assay as per the test method.

Table 3: Inter-day precision results.

Sample Name	Sample Absorbance	% Assay
1	0.268	99.80
2	0.269	100.17
3	0.265	98.68
4	0.267	99.43
5	0.268	99.80
6	0.270	100.54
Average	0.268	99.74
% RSD	0.64	0.64

Acceptance criteria: The % RSD from the six sample preparations should be no more than 2.0%. The individual % Assay should not less than 98.0% and not more than 102.0%.

3. Accuracy

Recovery: To determine the accuracy of the test method samples were prepared by spiking Atovaquone raw material with the equivalent amount of placebo at 50%, 100%, and 150% of the target concentration. Three samples were prepared at each concentration level. The average % recovery of Atovaquone was found to be within the limits.

Table 4: Accuracy results.

Sample No.	Spike Level (in %)	Amount added (mg)	Amount found (mg)	% Recovery	Mean % Recovery
1	50	5.00	5.03	100.56	100.81
2	50	5.00	5.07	101.31	
3	50	5.00	5.03	100.56	
1	100	10.00	10.02	100.18	100.56
2	100	10.00	10.06	100.56	
3	100	10.00	10.09	100.93	
1	150	15.00	15.19	101.31	100.98
2	150	15.00	15.16	101.06	
3	150	15.00	15.08	100.56	

Acceptance criteria

The mean recovery at each level should not be less than 98.0% and not more than 102.0%.

4. Specificity and selectivity

The analyte should have no interference from other extraneous components and be well resolved from them. The specificity of the method was evaluated regarding interference due to the presence of any other excipients.

The analyte should have no interference from other extraneous components and be well resolved from them. The specificity of the method was evaluated by comparing with blank solution and there is no interference found with the drug peak.

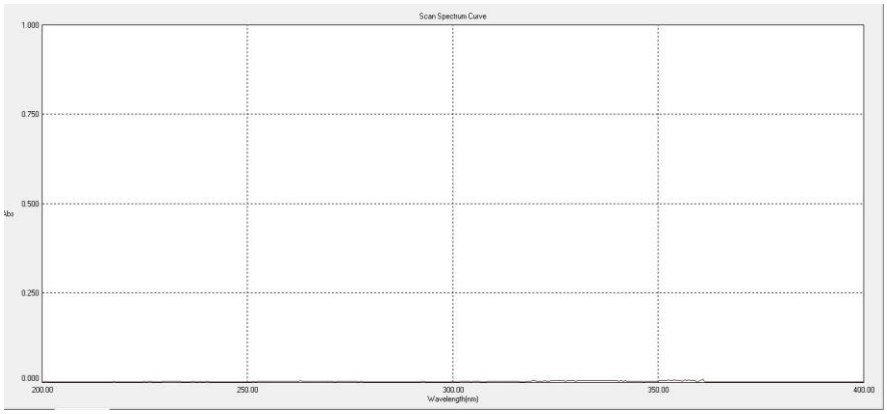

Figure 3: Blank spectrum.

5. %Assay

Standard solution preparation: (10µg/ml)

Pipette out 1 ml from the above stock solution into a 100 ml volumetric flask, and dilute up to the mark with methanol, mix well.

Sample solution preparation

Average weight of twenty tablets of Atovaquone (AtovaQuin) was calculated, an amount equivalent to 100 mg of Atovaquone was weighed and dissolved in 100 ml of methanol. Pipette out 1 ml from the above solution into a 100 ml volumetric flask, and dilute up to the mark with methanol.

CALCULATION

% assay of Atovaquone =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

Where:

AT = Absorbance of Atovaquone obtained with test preparation

AS = Absorbance of Atovaquone obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

% assay of Atovaquone =

$$\frac{0.987}{0.989} \times \frac{100}{100} \times \frac{100}{100} \times \frac{99.5}{100} \times \frac{254}{250} \times 100$$

= 0.99×1.016×99.5

= 100.08%

6. Limit of detection (lod) and limit of quantitation (loq)

The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations LOD = 3.3 σ/S and LOQ = 10σ/S, where σ is the standard deviation of the blank and S is the slope of the calibration plot.

DISCUSSION

The objective of the study was to develop and validate a simple, sensitive, and economical UV-Visible spectrophotometric method for estimating Atovaquone in pharmaceutical formulations, following ICH guidelines. Various solvents were tested, with methanol selected due to its good solubility and consistent absorbance. The maximum absorbance (λmax) was observed at 276 nm for a 10 µg/ml solution.

The method was validated for accuracy, precision, linearity, LOD, and LOQ. Recovery studies confirmed accuracy, with results ranging from 98% to 102%. Precision was evaluated through intra-day and inter-day studies, with %RSD values within the acceptable limit of 2%, indicating good reproducibility.

Linearity was observed in the concentration range of 2–10 µg/ml, with strong correlation coefficients. Optical characteristics, including Beer's law limits, molar absorptivity, slope, and intercept, were established. LOD and LOQ were calculated using standard deviation and slope values, meeting the required criteria.

Overall, the developed method is accurate, precise, and reliable for routine quality control analysis of Atovaquone in pharmaceutical products.

CONCLUSION

A simple and efficient UV spectrophotometric method was developed and validated for the estimation of Atovaquone in pharmaceutical formulations. Methanol was used as the solvent, and the drug showed maximum absorbance at 276 nm. The method was validated as per ICH guidelines for parameters including linearity, precision, accuracy, sensitivity, and specificity. Recovery studies confirmed high accuracy, with results within acceptable limits. Low inter-day and intra-day %RSD values indicated good precision. The method is accurate, precise, cost-effective, and easy to perform, making it suitable for routine analysis of Atovaquone in quality control laboratories without the need for complex or expensive equipment.

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