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Research Article

Development and Validation of Stability-Indicating RP-HPLC Method for Estimation of Atovaquone

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ABSTRACT

A rapid RP-HPLC method developed for determination of Atovaquone in API. Atovaquone was found to be degraded under different set of conditions as followed according to ICH guidelines and the degradants so formed along with Atovaquone are separated by using Thermo Hypersil BDS C_{18}, 250mm×4.6mm×5µm column using Buffer: Acetonitrile (20:80) as mobile phase, with a flow rate of 1.5ml/min with a detection wavelength of 283nm with a injection volume of 20µl. The method was validated for specificity, linearity, accuracy, robustness, and precision. The obtained results were indicating that the method is selective in analysis of Atovaquone in the presence of degradation products formed under various stress conditions.

Key words: Atovaquone, RP-HPLC, stability-indicating, Validation.

INTRODUCTION

Atovaquone is chemically trans 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4 naphthalene-dione and it is official in Indian pharmacopoeia. It is used to treat the Pneumocystis Pneumonia, toxoplasmosis and malaria.

MATERIALS AND METHODS

Chemicals and Reagents: Atovaquone was obtained as gift sample from Rainbow pharma training Lab, Hyderabad, Andhra Pradesh. We used HPLC grade acetonitrile, methanol, and water, AR grade ortho phosphoric acid.

Instrumentation: A HPLC (Waters, 2996) with PDA detector and Hypersil BDS C_{18} 250mm×4.6mm×5µm column was used. The HPLC system was equipped with Empower2 software for data processing. All the weighting's were done on single pan balance (Sartorius).

Chromatographic Conditions: The mobile phase containing Buffer: Acetonitrile (20:80) was found to resolve Atovaquone. Ortho phosphoric acid was used for pH adjustment of buffer to 3.15. The mobile phase was filtered through 0.45 nylon filter and then ultrasonicated for 30 min. The flow rate was set to1.5ml/min. The drug shows good absorbance at 283nm, which was selected as wavelength for further analysis.

Buffer preparation: Accurately transfer 1000ml of distilled water into 1000ml beaker and adjust P^H with ortho phosphoric acid to 3.15. Filter the solution through 0.45µm nylon filter.

Preparation of Mobile Phase

Filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 20:80 v/v

Preparation of diluent: HPLC grade methanol was used as diluent.

Preparation of Standard solution: Accurately weighed and transferred about 50 mg of Atovaquone standard into a 100ml clean dry volumetric flask add 30 ml of diluent, sonicated for 15 minutes and made up to the mark with diluent (this is standard stock solution).

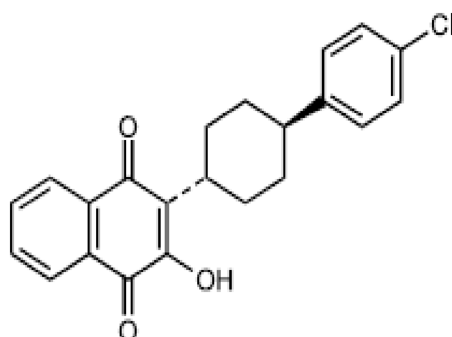

Fig.1. Chemical structure of Atovaquone

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Table.1: System Suitability Data

Parameter	Atovaquone
Tailing Factor	1.704
Theoretical Plates	14212
%RSD of Peak area	0.1

Table.2: Linearity Data

Level	Concentration (mg/ml)	Peak Area
50%	0.050	2945051
75%	0.075	4400905
100%	0.100	5873198
125%	0.125	7334958
150%	0.150	8809979
Slope	-	58655636
Intercept	-	7254.6
Correlation Coefficient	-	1.00

Transferred 5.0 ml of the above solution in to 25ml volumetric flask made up to the mark with diluent. Filter the solution through 0.45µm nylon filter.

Preparation of Sample solution: 20 Tablets of Atovaquone were weighed and powdered in glass mortar. Transferred the powder equivalent to the 50mg of active ingredient into a 100ml of volumetric flask add 30ml of diluent sonicate for 15 minutes with intermittent shaking and made up to the mark with diluent (this is sample stock solution).

Transferred 5.0 ml of the above solution in to 25ml volumetric flask made up to the mark with diluent. Filter the solution through 0.45µm nylon filter.

Preparation of Placebo Solution: Accurately weighed and transferred about 75mg of placebo powder into a 100 ml volumetric flask add 60 ml of diluent sonicate for 15 minutes with intermittent shaking and made up to the mark with diluent.

Transfer 5.0 ml of the resulting solution into a 50 ml volumetric flask and made up to the mark with diluent. Filter the solution through 0.45 µg nylon filter paper.

Method Validation

1) System Suitability/System Precision:

System Suitability was performed by injecting six replicate injections of standard solutions of Atovaquone at 100% level (100µg/ml) and expressed as %RSD of peak area.

2) Linearity: [1]Standard solutions of various conc. of about 80%-150% level were injected into the chromatograph and calibration curve was constructed by plotting peak area against concentration in µg/ml.

3) Specificity: To demonstrate that diluents and placebo are not interfering with analytic peak, solutions of blank, Atovaquone standard, sample and placebo were prepared individually and run the chromatogram. The peak purity of analyte peak should be not less than 0.999.

4) Precision: [2] Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the standard injections.

5) Accuracy: [3] %Recovery studies were carried out at three different levels of 50%, 100% and 150% of standard solution (i.e. Atovaquone API spiked to the placebo) in triplicate in each level.

6) Robustness: The robustness[2,4] of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate (±0.2ml/min), Buffer composition (±2%) which may differ but the responses were still within the specified limits.

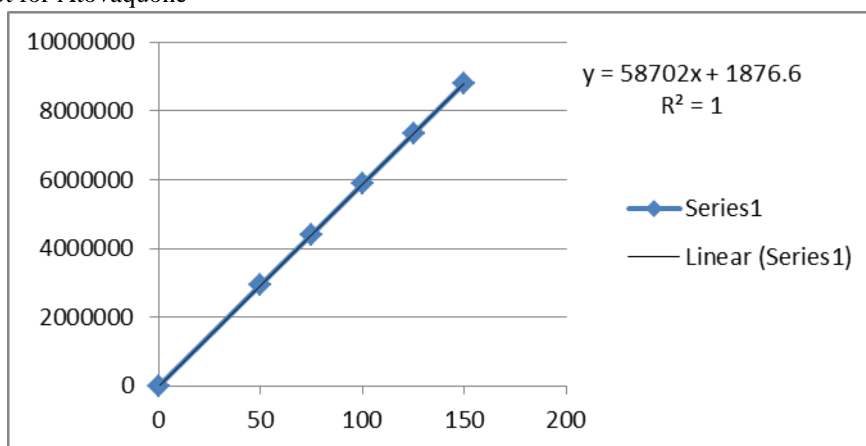
Forced Degradation Studies[5]: Photo Stability: Expose about 1000mg of powder in photo stability for 1.2 million Lux hours. Weigh accurately this powder equivalent to 50mg of Atovaquone into a 100ml volumetric flask add 60ml of diluent and sonicate for 15 minutes with intermittent shaking and made up to the mark with diluent. Filter the solution through 0.45µ nylon filter.

Acid Degradation: Weigh accurately about 50mg of sample and transfer into a 50ml volumetric flask and add 25ml of methanol and sonicate for 30 minutes and made up with methanol. Then add 10ml of 0.1N HCL and sonicate place it aside for 2-3hrs, then neutralize the solution with 10 ml of base and then transfer 5ml of the above solution into 25ml volumetric flask dilute with methanol and filter the solution through 0.45µ nylon filter.

Base Degradation: Weigh accurately about 50mg of sample and transfer into a 50ml volumetric flask and add 25ml of methanol and sonicate for 30 minutes and made up with methanol. Then add 10 ml of 0.1N NaOH and sonicate place it aside for 2-3hrs, then neutralize the solution with 10ml of acid and then transfer 5ml of the above solution into 25ml volumetric flask dilute with methanol and filter the solution through 0.45µ nylon filter.

Peroxide Degradation: Weigh accurately about 50mg of sample and transfer into a 50ml volumetric flask and add 25ml of methanol and sonicate for 30 minutes and made up with methanol. Then add 0.1 ml of 10% H_2O_2 and sonicate place it aside for 2-3hrs, then neutralize and then transfer 5ml of the above solution into 25ml volumetric flask dilute with methanol and filter the solution through 0.45µ nylon filter.

Fig 2: Linearity plot for Atovaquone



The chromatograms for specificity were shown in Fig.3, 4 and 5.

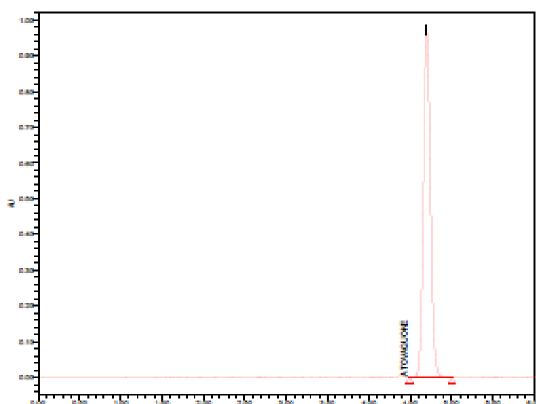


Fig.3.Standard chromatogram

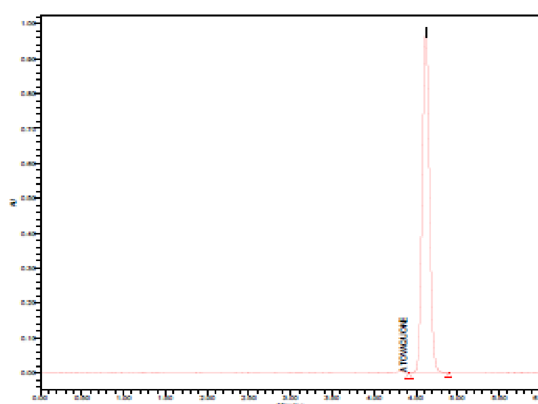


Fig.4. Sample chromatogram

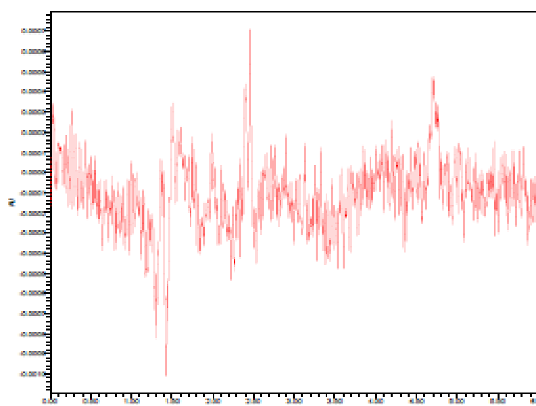


Fig.5.Blank chromatogram

RESULTS AND DISCUSSION

Optimization of the mobile phase was based on asymmetric factor and peak area obtained for Atovaquone. The Mobile phase Acetonitrile: Buffer (80:20) was found to be satisfactory and gave symmetric peak for Atovaquone. Results were summarized in Table.no.1.

The correlation coefficient was found to be 1.0 and results were summarized in Table.2.

Precision was determined and the results are represented in the form of %RSD for assay and results were given in Table-3.

The percentage mean recovery was found as 99.32%. The results were summarized in Table.4.

As part of the robustness study deliberate changes in the flow rate and buffer composition was made to the impact on the method. Retention time was significantly changed

Table.3: Repeatability Data

Injection No.	%Assay
1	98%
2	98%
3	98%
4	100%
5	99%
6	99%
Mean	98
%RSD	0.87

Table.4: Accuracy Data

Drug	%Level	Amount added (mg)	Amount found (mg)	Mean %Recovery	%RSD
	50%	0.059	0.059	100%	1.1
Atovaquone	100%	0.113	0.111	99%	0.1
	150%	0.164	0.160	98%	0.9

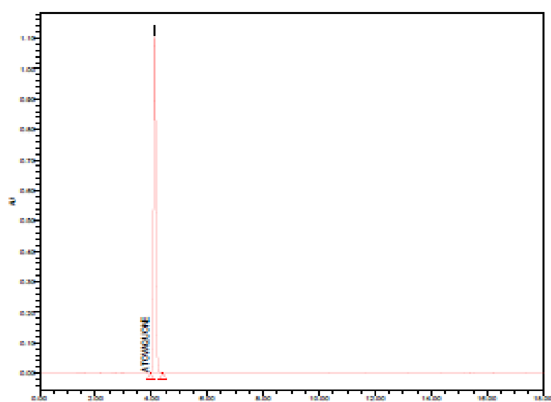


Fig.6: Acid Stress condition

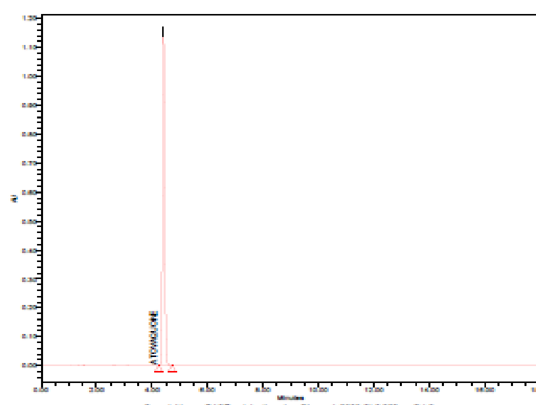


Fig.7: Base stress condition

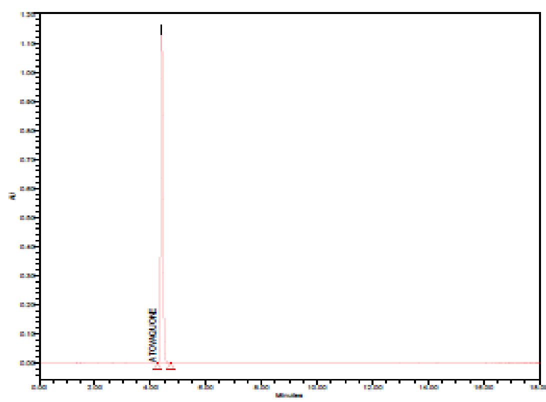


Fig.8: Peroxide stress condition

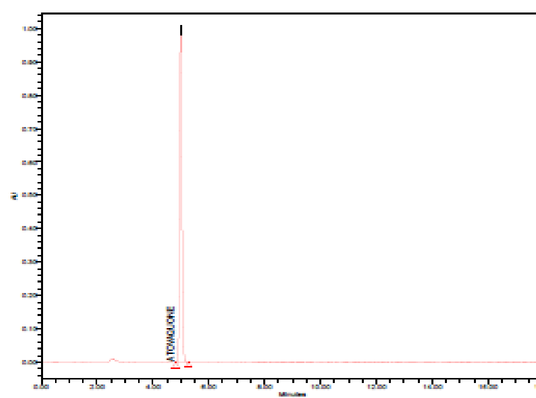


Fig.9: Thermal stress condition

Table.5: Robustness Data

Parameter	RT of Atovaquone
Actual	4.919
High Flow Rate : 1.7ml/min	3.893
Low Flow Rate : 1.3ml/min	5.080
High Temperature : 50^0c	4.346
Low Temperature : 40^0c	4.436

Table.6. Forced Degradation Data

Treated Parameter	RT	Peak Purity Index
As Such	4.919	1.0000
Photo Stability	4.992	1.0000
Acid Heat Treatment	4.119	1.0000
Base After Heat	4.411	1.0000
Oxidative Treatment After Heat	4.411	1.0000

but within the acceptance limit and results given inTables.5.

Result for Force Degradation Studies: The study showed that slight degradation observed when treated with acid, base, peroxide after heating and photo stability conditions. The peak purity index for known impurity and drug was found to be within acceptance criteria.i.e. Less than 0.990 and results were given inTables.6 and the chromatograms for forced degradation were shown in Fig.6, 7, 8 and 9.

CONCLUSION

It can be concluded that the proposed RP-HPLC method is accurate, precise, sensitive, specific, robust and reproducible for the analysis of Atovaquone with less tailing and is also economical.

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