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DEVELOPMENT AND VALIDATION OF ZERO AND FIRST ORDER DERIVATIVE UV-SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ATOVAQUONE IN BULK AND IN FORMULATION

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ABSTRACT

Two simple, sensitive, precise and economical UV- spectrophotometric methods have been developed for the determination of Atovaquone in suspension. Method A is simple and direct UV spectrophotometric method and is based on determination of Atovaquone in methanol and acetate buffer in the ratio 70:30 at 277.50 nm. Method B is first order derivative spectrophotometric method and involved in the estimation of Atovaquone in same solvent mixture using the first-order derivative technique at 267.50 nm as maxima and 297 nm as minima. Calibration curve was prepared by plotting the absorbance difference between maxima and minima versus concentration. Linearity was obtained in the concentration range of 2-10µg/ml for both the methods. These methods were successfully applied to pharmaceutical formulations because no interferences from formulation excipients were found. The suitability of these methods for the quantitative determination of Atovaquone was proved by validation. The proposed methods were found to be simple, sensitive, accurate, precise, rapid and economical for the routine quality control application of Atovaquone in pharmaceutical formulations.

KEY WORDS

Atovaquone, methanol-ammonium acetate buffer, zero order, first order derivative spectrophotometric, validation, UV spectrophotometric method.

INTRODUCTION

Atovaquone [**Figure 1**] is a potent hydroxyl naphthoquinone with approved use in the USA, Canada and several European countries for the treatment of Pneumocystis carinii pneumonia in acquired immunodeficiency syndrome (AIDS) patients intolerant to Trimethoprim / sulfamethoxazole. Its potent antiprotozoal activity against Plasmodium, Pneumocystis and Toxoplasma had prompted further investigations including clinical trials for T.gondi encephalitis in AIDS patients. To date, the assays published for atovaquone are limited to complex gas chromatographic methods and high-performance liquid chromatography (HPLC) methods with multiple sample preparation and extraction procedures.

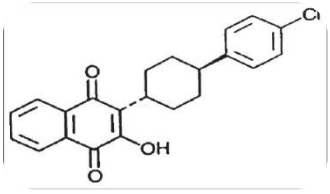

Figure 1: Chemical structure of atovaquone Trans-2-[4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1, 4-naphthoquinone.

MATERIALS AND METHODS

Instrument:

A Lab India model Double beam spectrophotometer with UV WIN software was used for all the spectrophotometric measurements and treatment of data. Zero-order absorption spectra were traced in 1cm quartz cells over the range of 200-400 nm. Calibrated Class 'A' volumetric glass wares were used.

Preparation of standard stock solution:

About 100 mg of the drug was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in about few ml of solvent mixture .The volume was then made up to the mark with the same solvent mixture.

Preparation of working standard solution:

Ten milliliters of this stock solution was transferred to a 100 ml volumetric flask and further diluted up to the mark with solvent mixture. This solution contained 100 µg of drug per milliliter of the solution.

DEVELOPMENT OF METHODS

Method-A: Zero order spectroscopic method:

The solutions were scanned in the range of 400-200nm, and the peak was observed and gives maximum absorbance at 277.50nm. So, the wavelength selected of analysis of drug is 277.50nm. The drug followed the Beer's-Lambert's law in the range of 2-10µg\ml.

Method-B: First order spectroscopic method:

The standard drug solution was diluted so as to get the final concentration in the range of 2-10µg\ml and scanned in the first order derivative spectra. The first order derivative spectra showed a maxima and minima at 267.50 and 297nm respectively. The amplitude of absorbance was measured at 267.50 nm (maxima) and at 297nm (minima) and was plotted against concentration to give calibration curve and regression equation was calculated. The amplitude was linear in the concentration range of 2-10µg\ml.

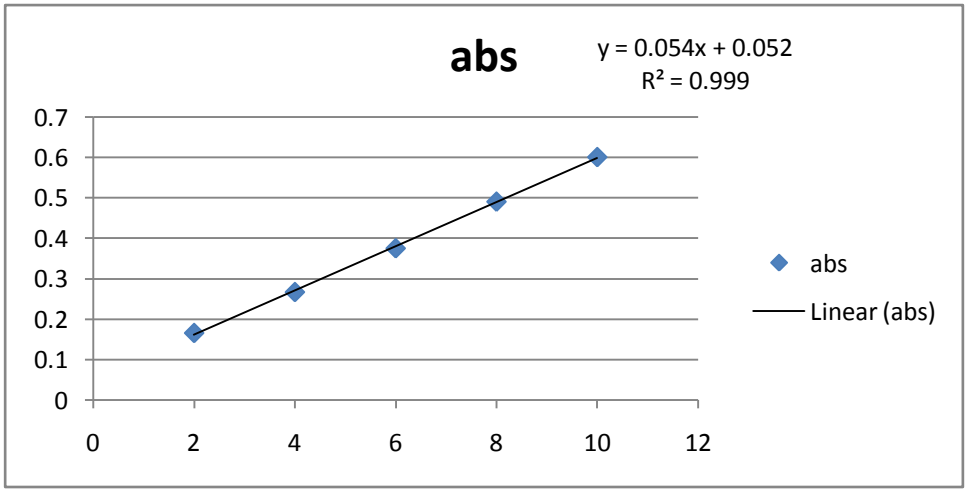

Figure-1: Zero order calibration curve.

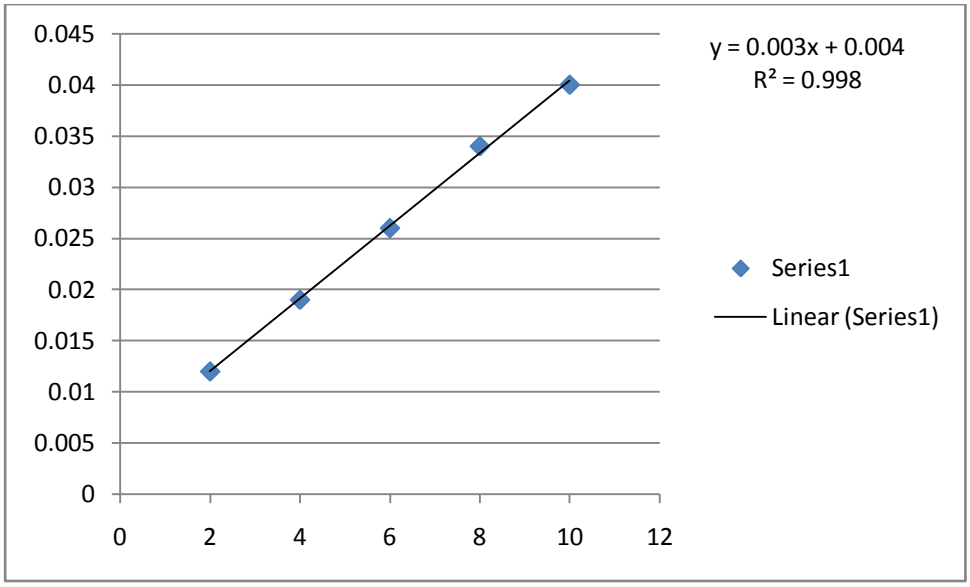

Figure-2: First order calibration curve

Validation of the proposed methods

The proposed method is validated according to International Conference on Harmonization (ICH) guidelines.

Linearity:

Calibration curves for Atovaquone were plotted over a concentration range of 2-10µg\ml for all the methods. Accurately measured standard working solutions of Atovaquone (0.2, 0.4, 0.6, 0.8, 1.0ml) were transferred to a series of 10ml volumetric flasks and diluted up to the mark by diluent. Absorbance was measured at a wavelength 277.50nm and was plotted absorbance versus concentration gives calibration curve for method-A. First derivative curves shows maxima at 267.50nm and minima at 297nm.The calibration curve of amplitude against concentration of the drug showed linearity for method-B.

Accuracy:

The accuracy of the method was performed by calculating recovery of Atovaquone by the standard addition method. Known amounts of standard solutions of Atovaquone were added at 50, 100, 150% levels of prequantified sample solutions of Atavaquone. At each level of the amount three determinations were performed. The amount of Atovaquone was estimated by applying obtained values to regression equation. Calculate the amount found and amount added for Atovaquone and calculate the individual recovery and mean recovery values.

Table-1: Results of accuracy:

Zero order derivative method					
Accuracy level	**Absorbance**	**Amount added**	**Amount found**	**%Recovery**	**Mean recovery**
50%	0.216	5.0	4.96	99.3%	
100%	0.433	10.0	9.95	99.5%	99.6%
150%	0.653	15.0	15.0	100.1%	
First order derivative method					
50%	0.006	5.0	4.99	99.8%	
100%	0.012	10.0	9.98	99.8%	99.8%
150%	0.018	15.0	14.9	99.8%	

Table-2: Results of intra and inter day precision:

Parameters	Intraday precision		Interday precision	
	SD	%RSD	SD	%RSD
Zero derivative	0.0007	0.16	0.0013	0.29
First derivative	0.00005	0.45	0.00004	0.37

Table-3: Assay results for determination of Atovaquone in pharmaceutical formulation:

Parameters	Label claim	Drug content%	%RSD
Zero order	750mg /5 ml	100.2%	0.132
First order	750mg/5 ml	100.03%	0.47

Method precision (% repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions of Atovaquone without changing the parameters for the methods. The repeatability was expressed in terms of relative standard deviation.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed methods was performed by analyzing the corresponding responses three times on the same day and on three different days with same dimensions.

Limit of detection and Limit of quantification:

The Limit of detection (LOD) and Limit of quantification (LOQ) of a drug were derived by visually or by calculating the signal-to-noise (i.e., 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD=3.3\infty/S$$

$$LOQ=10\infty/S$$

Where ∞= the standard deviation of the response

S= slope of the calibration curve

Estimation of Atovaquone in pharmaceutical formulation:

Pharmaceutical formulation of Atovaquone was purchased from local pharmacy. Sample was taken having the drug equivalent to label claim and the solutions were prepared as earlier. Then these solutions were analyzed by two methods. The nominal content of the formulation was determined either form calibration curve or the regression equation.

Results and discussions:

Method –A is simple uv- spectrophotometric method. In this method the simple UV spectrum of Atovaquone in methanol was obtained which exhibits absorption maxima at 277.50nm. The calibration curve was linear in concentration range of 2-10µg\ml. Method-B is the first derivative spectroscopic method. Maxima occur at 267.50nm and minima occur at 297nm. The calibration curve was linear in concentration ranges of 2-10µg\ml. The proposed methods were found to be simple, sensitive, rapid, accurate, precise and economic fir routine analysis of Atovaquone in pharmaceutical formulation. Accuracy was determined by calculating the recovery, and the mean. Precision was calculated as repeatability (relative standard deviation) and intra and inter day variation (%RSD) for Atovaquone. The %RSD values for Atovaquone was found to be less than 2, which indicates the method is precise. LOD values for Atovaquone were found to be 0.06&0.078 µg\ml for method A&B respectively. LOQ values were found to be0.186&0.25 µg\ml for methods A&B respectively indicates sensitivity of the proposed methods.

The methods were successfully used t o determine the amount of Atovaquone in pharmaceutical formulation. The results obtained are in agreement with the corresponding labeled amount. By observing the validation parameters, the methods were found to be precise, accurate and sensitive. Hence these methods can be used for routine analysis.

Table-4: Regression analysis data and summery of validation parameters for the proposed methods:

Parameter	Zero order	First order
Absorption maxima and minima	277.50	267.50 and 297
Beer's- Lambert's range (µg|ml)	2-10	2-10
Regression equation y=mx+c	Y=0.054x+0.052	Y=0.003x+0.004
Slope(m)	0.054	0.003
Intercept(c)	0.052	0.004
Correlation coefficient(r2)	0.999	0.998
Mean recovery %	99.6	99.8
Precision (%RSD)	0.16	0.45
Intermediate precision	0.29	0.37
LOQ(µg/ml)	0.06	0.078
LOD(µg/ml)	0.186	0.25

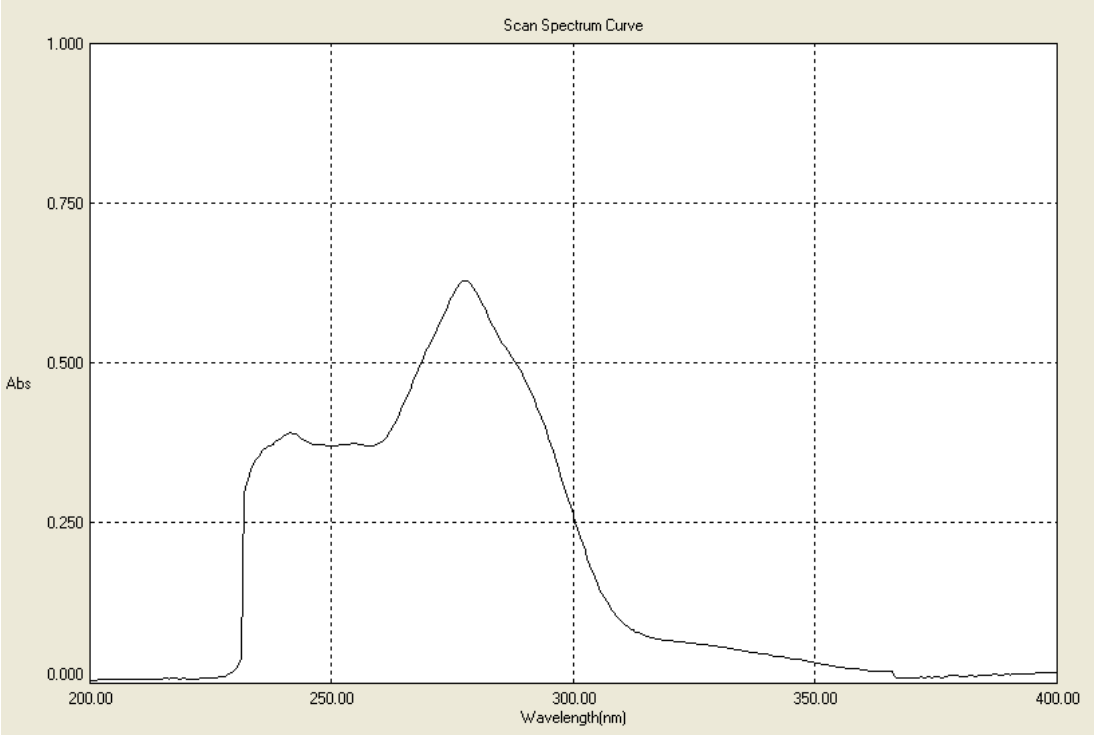

Figure-3: Zero order derivative and overline spectrum of Atovaquone

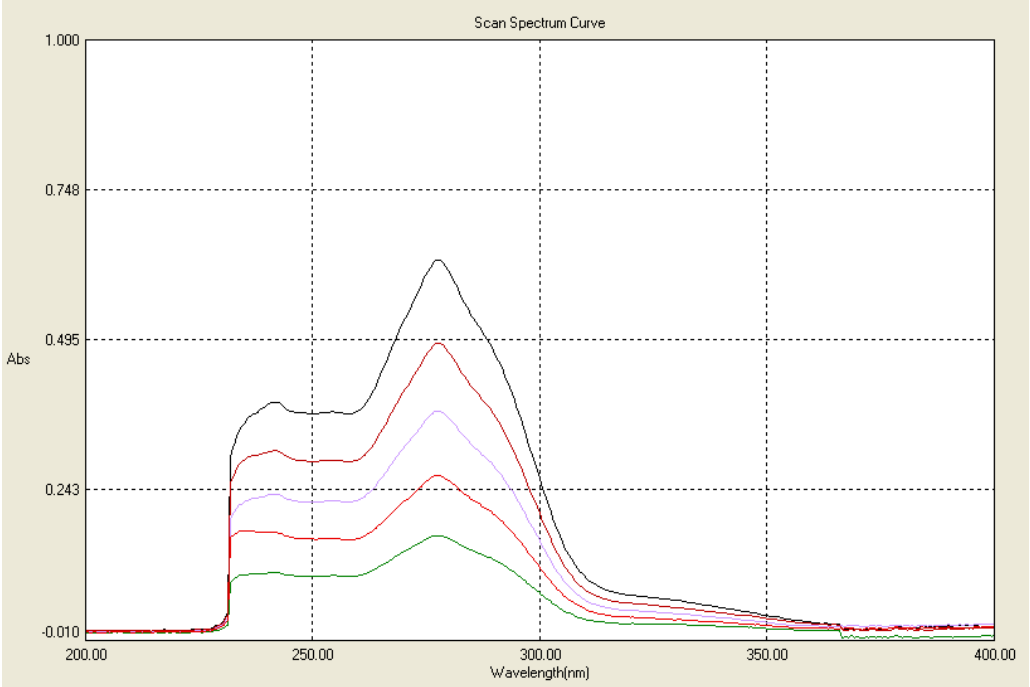

Figure-4: zero order derivative and overlain spectrum of atovaquone:

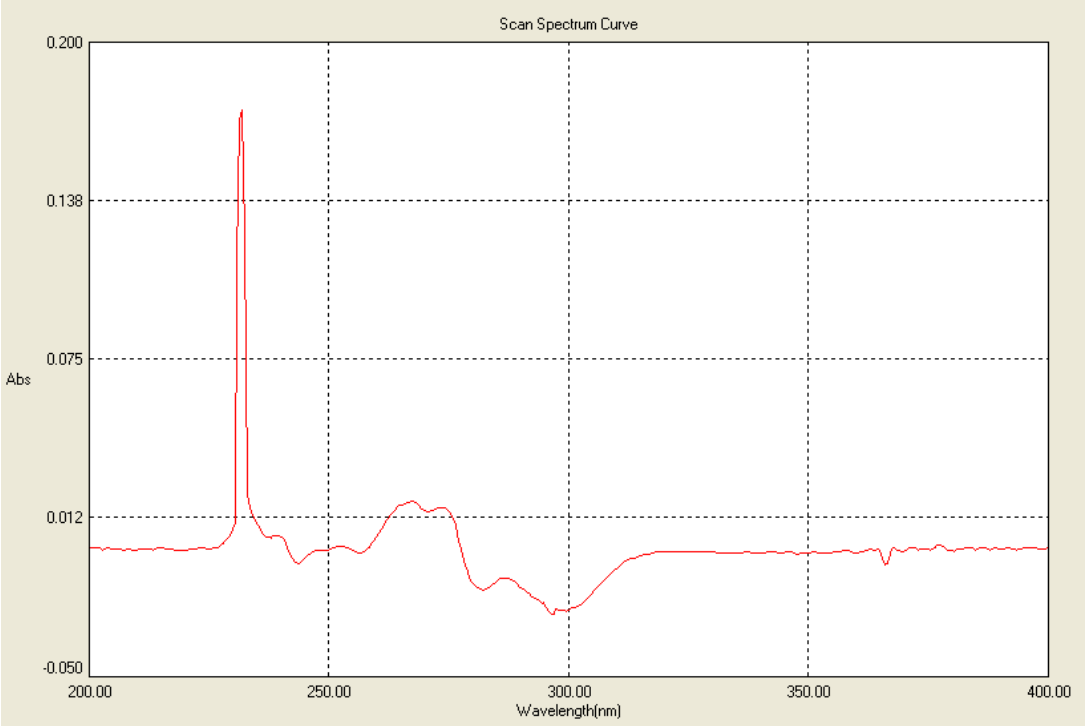

Figure-5: first order derivative spectrum of Atovaquone:

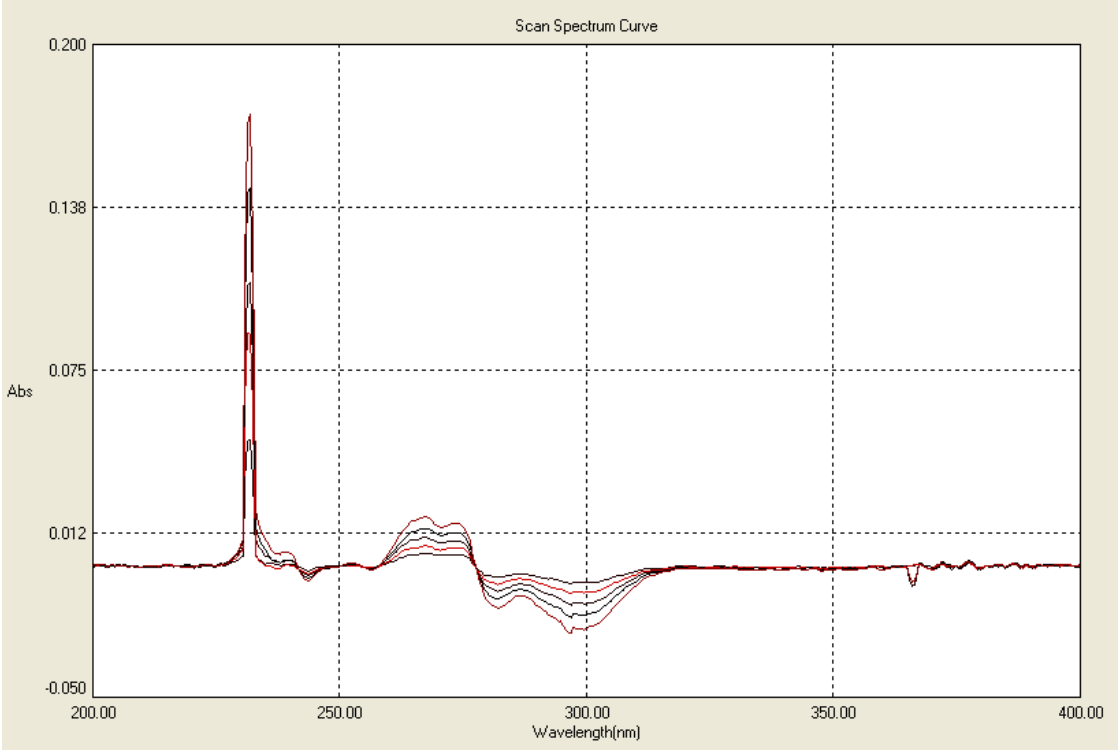

Figure-6: First order derivative and overlain spectrum of Atovaquone

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