www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Development and validation of simple spectrophotometric, first and second derivative spectroscopy for estimation of Atovaquone in bulk and pharmaceutical formulations

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ABSTRACT

A sensitive, selective and well validated spectrophotometric method using Tetrahydrofuran and Chloroform have been proposed for the determination of Atovaquone in pharmaceutical formulations which is widely used as antimalarial agent. The developed spectrophotometric method is simple, rapid, precise, accurate, reliable and economical when compared to other methods. The method was also applied for first and second derivative spectroscopy. The method shows better results in terms of accuracy, precision and linearity over a range of 20-100µg/ml for Tetrahydrofuran and 10-50 µg/ml for Chloroform. The limit of detection in Tetrahydrofuran and Chloroform are observed as 2 µg /ml and 5 µg/ml, the limit of quantification in Tetrahydrofuran and Chloroform are observed as 6 µg/ml and 15 µg/ml respectively. The %RSD is less than 2% in Chloroform and less than 1.5% in Tetrahydrofuran for precision. And the recovery is 99-101% in both the solvents. As a result the above method can be applied for bulk and finished product of Atovaquone.

Keywords: Atovaquone; Anti malarial; Chloroform; Tetrahydrofuran; UV spectroscopy.

INTRODUCTION

Atovaquone, (trans-2-(4-(4- chlorophenyl) cyclohexyl)-3- hydroxy-1,4-naphthalene dione) is a hydroxy naphthaquinone derivative used for treatment of malaria in adults. Atovaquone is freely soluble in N-methyl-2-pyrrolidone, tetrahydrofuran and chloroform. Atovaquone is a hydroxyl naphthaquinone derivative & an analog of ubiquinone, a parasite mitochondrial electron carrier which is a co-factor of the dihydro -- orotate dehydrogenase. Atovaquone acts by the inhibition of the parasitic mitochondrial electron transport (John martin, 2009). The substances were combined in the new antimalarial drug malarone, developed by Glaxowellcome & the effect of medium chain triglycerides was studied by Roland et al. (Bergquist Y.2000, 2006)

A few methods have been reported to estimate Atovaquone levels in biological fluids (Rolan P.E 1997). LC-UV methods have been reported for the separation of the components present in anti malarial drug combination (Almeida A.M, 2002) and also for the determination of atovaquone in plasma and for whole blood (Rolan P.E, 1994). Several other quantitative LC-UV method have also been reported (Oda Y. Huang, 1999). Dunay and colleagues published a pharmacokinetic study in mice using mass spectrophotometric detection and determined a limit of quantification at 617,000mg ml^{-1} and 51,000mg kg^{-1} for mouse serum and brain tissue respectively (Smith R.K, 1999). The present study reports on a newly developed and validated liquid chromatographic tandem mass spectrophotometric (LC-MS-MS) method for the determination of Atovaquone concentration in human plasma using chlorothalidone(2-chloro-5-(1-hydroxy-3-oxo-2H-isoiindol-yl)benzene sulfonamide) as internal standard (Satish Gangaram pingale 2009). High performance liquid chromatographic method has been developed for the estimation of atovaquone in human plasma (JosephL Woolley, 1994, Bergqvist Y, 2000, and Petrie M.Rainey, 1996)

The developed UV- spectrophotometric method which is easy to handle and requires less time for the analysis. It is also a simple, highly rapid and economic friendly method.

MATERIALS AND METHODS

Instruments: Spectrophotometric measurements were made on ELICO single beam spectrophotometer with a fixed slit width of 2nm coupled with spectra treats soft ware.

Chemicals: The chemicals used are Chloroform ($CHCl_3$) and Tetrahydrofuran (THF) obtained from (SD Fine Chemicals Limited, Mumbai) and Atovaquone reference standard (AURABINDO pharmaceuticals, Hyderabad, India) having a potency of 99.8%.

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Received on: 05-03-2011
Revised on: 15-04-2011
Accepted on: 16-04-2011

Solutions: Stock solution 1mg/ml is prepared by dissolving pure drug in $CHCl_3$and THF individually. From stock solution 0.1ml is taken and made upto 10ml with solvents and dilutions were made according to range.

PROCEDURE

Determination of Atovaquone by simple, first and second derivative spectrophotometry:

The absorption spectrum of pure Atovaquone was recorded between 200nm-400nm for spectrophotometric determination and calibration graph was also obtained. The λ_{max} was obtained at 288nm for chloroform and 252nm for tetra hydro furan respectively.

The first and second derivative spectra were plotted with delta lambda 2nm and scaling factor 10. Calibration graphs were obtained at the selected wave length of the first and second derivative spectra with the aim of best linearity and maximum absorption.

VALIDATION

Selectivity/specificity

A method is said to be specific when it produces a response only for a single analyte. Selectivity is the ability of the method to produce a response for the analyte in the presence of other interferences, in order to prove that the method chosen was specific and selective.

Sensitivity

Limit of detection (LOD) and Limit of quantification (LOQ) were calculated according to the 3:1 (S/N) and 10:1 (S/N) criterions respectively, where S is the signal of the sample and N is the noise of the corresponding curve.

Linearity and range

Linearity of the concentrations was taken in the range of 10-50µg/ml for chloroform and 20-100µg/ml for tetra hydro furan respectively.

Accuracy

Accuracy of proposed method from exipients was determined by recovery experiments. Recovery experiments were carried out in three levels of concentration. The amounts of standard recovered were calculated in the terms of mean recovery with the upper and lower limits of % relative standard deviation.

Precision

It is expressed as the percentage coefficient of variation (%CV) which is calculated as per the following expression:

%CV= (standard deviation /mean)*100

Intraday precision

It was determined by calculating the %coefficient of variation (%CV) of the results obtained in the same day.

Inter day precision

It was determined by calculating the percentage coefficient of variation (%CV) of the results obtained

Table 1: System suitability parameters

parameters	THF			Chloroform		
	Zero order	First derivative	Second derivative	Zero order	First derivative	Second derivative
Linearity and range (µg/ml)	10-50	10-50	10-50	20-100	20-100	20-100
Equation	Y=0.0056x +0.0007	Y=0.0095x+ 0.0795	Y=-0.0078x+ 0.0688	Y=0.0476x+ 0.2004	Y=0.048x+ 0.0037	Y=0.0034x+ 0.104
R^2	0.9975	0.9935	0.9956	0.9948	0.9938	0.9999
LOQ	10	10	10	15	15	15
LOD	3	3	3	5	5	5

Table 2: Precision studies in chloroform

Parameters	Chloroform								
	Zero derivative			First derivative			Second derivative		
	LQC (µg/ml)	MQC (µg/ml)	HQC (µg/ml)	LQC (µg/ml)	MQC (µg/ml)	HQC (µg/ml)	LQC (µg/ml)	MQC (µg/ml)	HQC (µg/ml)
	10	30	50	10	30	50	10	30	50
Mean	10.1783	30.27	50.14	10.13	30.13	50.18	10.21	30.09	50.09
SD	0.1824	0.1900	0.1220	0.1563	0.09	0.2044	0.2006	0.1602	0.4179
RSD	1.7927	0.6279	0.2433	1.5425	0.3290	0.4073	1.9635	0.5323	0.8343
%NOMINAL	101.78	100.90	100.29	101.38	100.45	100.37	102.18	100.31	100.19
N	6	6	6	6	6	6	6	6	6

Table 3: Precision studies in tetrahydro furan

Parameters	Tetra hydro furan								
	Zero derivative			First derivative			Second derivative		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
	20 µg/ml	60 µg/ml	100 µg/ml	20 µg/ml	60 µg/ml	100 µg/ml	20 µg/ml	60 µg/ml	100 µg/ml
MEAN	19.91	59.93	99.96	20.06	60.13	99.9	19.98	60.07	100.08
SD	0.2284	0.3945	0.2451	0.2955	0.5370	0.5859	0.3005	0.4309	0.2831
RSD	1.1475	0.6582	0.2452	1.4579	0.8931	0.5860	1.5013	0.7174	0.2828
%NOMINAL	99.55	99.89	99.96	100.33	100.22	99.98	99.92	100.11	100.08
N	6	6	6	6	6	6	6	6	6

Table 4: Recovery in chloroform

	Concentration taken (µg/ml)	Concentration added (µg/ml)	Recovered concentration (µg/ml)*	% recovery
Level – 1	10	10	19.998±0.276	99.99
Level – 2	30	10	40.365±0.216	100.91
Level – 3	50	10	60.588±0.372	100.98

* All values include mean±SD

Table 5: Recovery in tetrahydrofuran

	Concentration taken (µg/ml)	**Concentration added (µg/ml)**	**Recovered concentration (µg/ml)***	**% recovery**
Level – 1	20	10	29.878±0.23	99.59
Level – 2	60	10	71.365±0.154	101.95
Level – 3	100	10	109.588±0.302	99.62

* All values include mean±SD

over at least two days.

RESULTS & DISCUSSION

System suitability

System was evaluated for reproducibility by injecting six replicates of Atovaquone (1mg/ml) dilution. The coefficient of variation obtained was. The results obtained are given in Table 1. System was suitable for the determination of Atovaquone because the results were reproducible for the analyte.

Sensitivity

The limit of detection value for chloroform and tetra hydro furan were obtained as 5µg/ml and 2µg/ml. from this the limit of quantification determined as 15µg/ml for chloroform and 6µg/ml for THF.

Linearity

By following the linearity Zero, first and second derivative spectroscopy were determined. From this R^2 values are obtained as 0.9975, 0.9935, and 0.9956 for zero, first and second derivatives of chloroform and 0.9948, 0.9938, 0.9999 for zero, first and second derivatives of tetrahydrofuran respectively and the values are given in Table no.1 and in Fig 5-10.

Accuracy

The mean absolute recovery of Atovaquone in both the solvents is 99-101%.

Precision

By the precision studies the relative standard deviation values were obtained as less than 2% for chloroform and less than 1.5% for THF the values were given in Table 2 and 3 respectively.

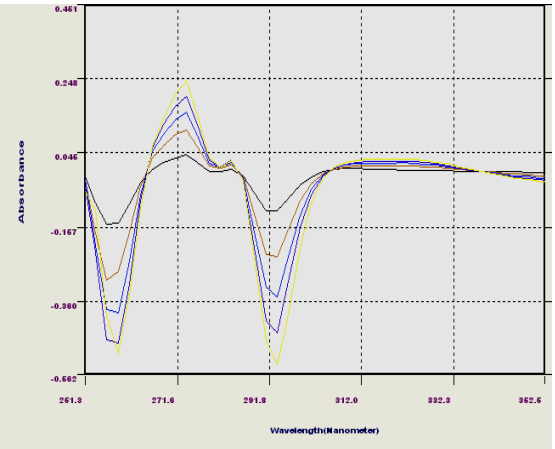

Figure 1: First derivative spectrum of chloroform

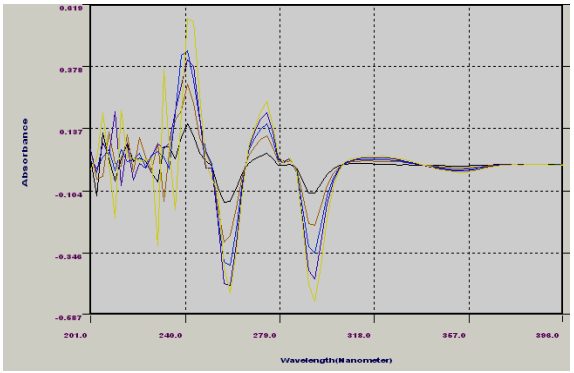

Figure 2: First derivative spectrum of THF

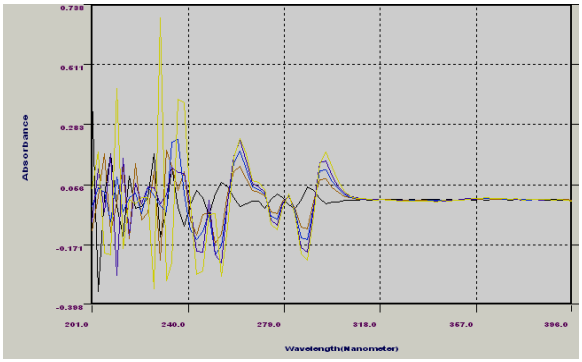

Figure 3: Second derivative spectrum of THF

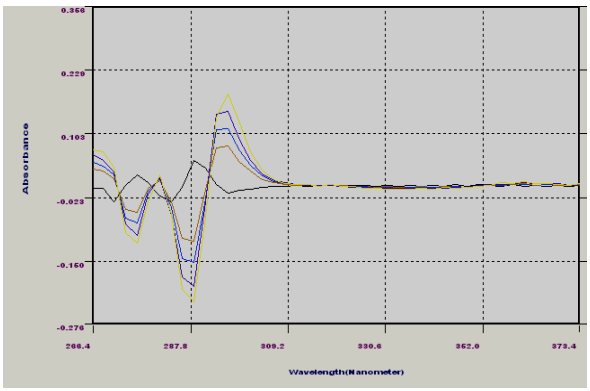

Figure 4: Second derivative spectrum of chloroform

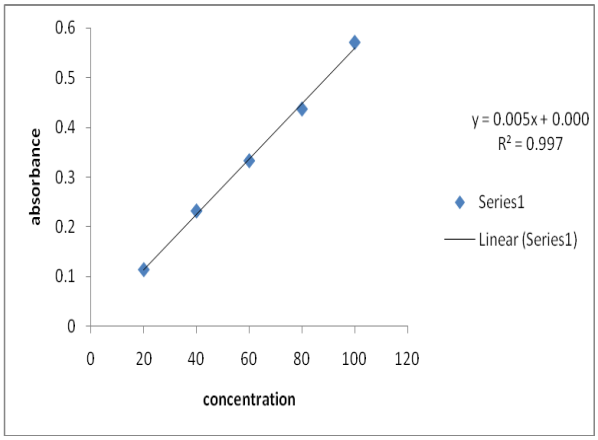

Figure 5: Zero order curve for tetra hydro furan at λ_{MAX} 252

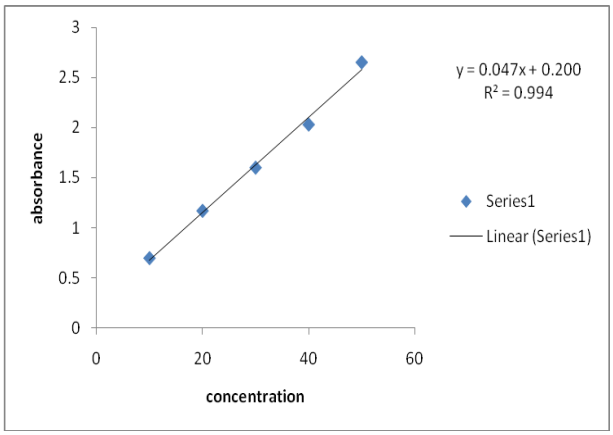

Figure 6: Zero order curve for chloroform at λ_{MAX} 288

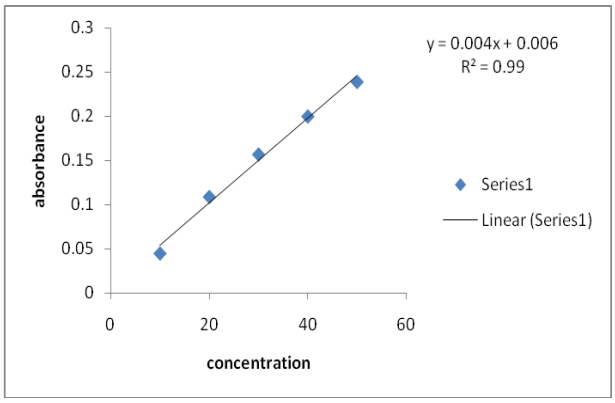

Figure 7: First order curve for chloroform

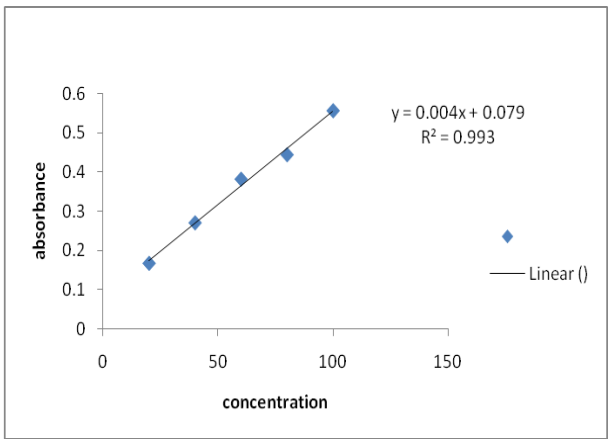

Figure 8: First order curve for tetra hydro furan

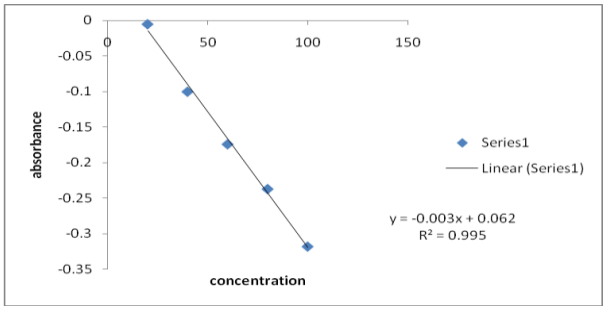

Figure 9: Second order curve for tetra hydro furan

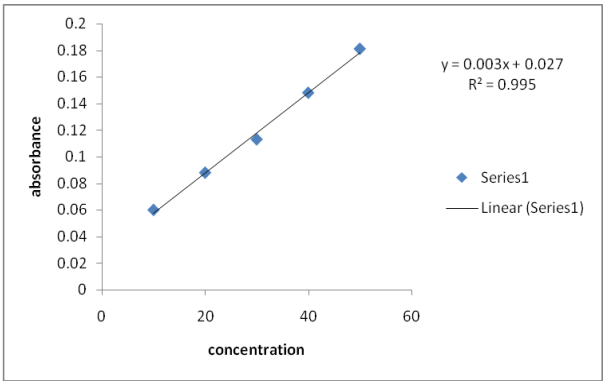

Figure 10: Second order curve for chloroform

CONCLUSION

Finally with the above results it is concluded that the developed method is simple, rapid and accurate which can be applied to the estimation of atovaquone in bulk and pharmaceutical formulations with minimum errors.

ACKNOWLEDGEMENT

We are indebted to Management of Sri Siddhartha Pharmacy College, Nuzvid for providing adequate lab facilities and excellent infrastructure for the execution of this work into a grandiose one.

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