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Development and validation of q-absorbance ratio method for simultaneous estimation of arterolane maleate and piperaquine phosphate in pharmaceutical dosage form

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ABSTRACT

New Spectrophotometric Q-Absorbance Ratio method has been developed for the simultaneous estimation of Arterolane maleate and Piperaquine phosphate in tablet dosage form. UV spectrophotometric method, methanol was used as a solvent.

Objective

To develop simple, accurate, linearity, precision and reproducible UV Spectroscopic method for Arterolane maleate and Piperaquine phosphate in routine analysis.

Method

Aliquots of stock solution were further diluted with methanol to get working solution of 2.5-8.75µg/ml for Arterolane maleate and 12.5-43.5 µg/ml for Piperaquine phosphate working standards were scanned between 200 - 400 nm which shows the maximum absorbance at 276nm.

Results

The Iso -absorptive point was found to be 242 nm. Calibration curve were linear over a concentration range of 2.5-8.75 µg/ml for Arterolane maleate and 12.5- 43.5 µg/ml for Piperaquine phosphate. Accuracy of method was determined through recovery studies which were found 99.36%-101.22% for Arterolane maleate and 99.46%-100.80% for Piperaquine phosphate. Method was found to be reproducible with relative standard deviation for intra-day and inter-day precision to be < 1.5%.

Conclusion

This method was found to be simple, Accurate, precise and reproducible. The proposed UV method can be applicable for the simultaneous estimation of both the drugs in tablet dosage form.

Keywords: Arterolane maleate, Piperaquine phosphate, Q-Absorbance Ratio method, Analytical method validation, Methanol.

INTRODUCTION

Arterolane maleate (AM) is chemically known as [(N-(2-amino-2-methylpropyl)-2-cis-dispiro (admantane-2, 3'-[1, 2, 4] trioxolane-5, 1"-cyclohexan)-4"-yl] acetamide: maleate. Arterolane maleate is synthetic peroxide which acts as anti-malarial agent by rapid acting as blood

schizonticides against all blood stages of plasmodium falciparum without having effect on liver stages. Its molecular structure is uncommon for pharmacological compounds in that it has both an ozonide group and an adamantane substituent [1].

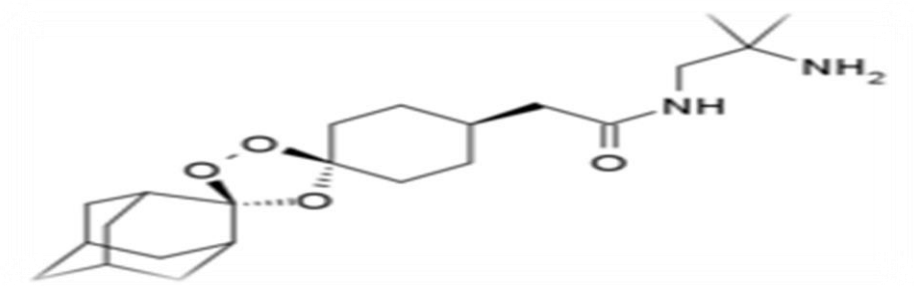

Fig. 1: Chemical Structure of Arterolane maleate

Piperaquine phosphate (PQP) is chemically known as 1, 3-bis [4-(7-chloroquinoline-4-yl) piperazin-1-yl] propane: Phosphoric acid. It is a bisquinoline of an antimalarial drug, used as a prophylaxis and which shows good activity against chloroquine-resistant plasmodium strains [2,3]

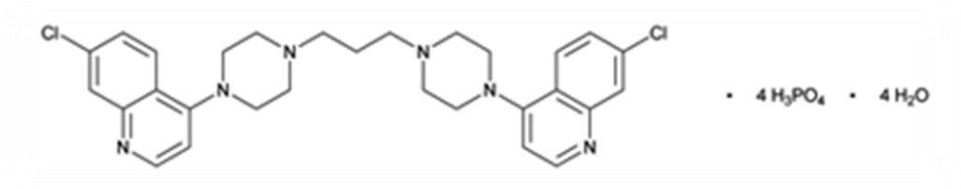

Fig. 2: Chemical Structure of Piperaquine phosphate

Combination of AM and PQP is available in tablet dosage form in the ratio of 150:750 mg. AM is official in Indian Pharmacopoeia 2014 [4]. PQP is official in United State Pharmacopoeia [3]. But combination of these drugs is not official in any pharmacopoeia. The combination of AM and PQP has been approved by Central Drug Standard Control Organization (CDSCO) on dated 19/10/2011 [5]. Very few methods like HPLC [6-8], Capillary zone electrophoresis[9] , LC-MS[10-13] have been reported as a single or in combination with other drugs .Literature reveals that there is no single UV spectroscopic method for AM and PQP in pharmaceutical dosage form. So, the present study aim at development of a simple, accurate, precise method for simultaneous estimation of AM and PQP in pharmaceutical dosage form by Q-Absorbance Ratio method.

MATERIALS AND METHODS

Apparatus and Instrument

Double beam UV- visible spectrophotometer (Shimadzu, model 1800 Spharmaspec) having two matched quarts cells with 1 cm light path, Electronic analytical balance, BL-220H, pH meter, LI- 610.All instruments and glass wares were calibrated.

Reagents and Materials

Arterolane maleate and Piperaquine phosphate (gifted by Gitar Laboratories, Ahmadabad, India) SYNRIAM Tablet (procured from local market.), Methanol AR (Merck Pvt. Ltd, India)

Preparation of standard stock solution

10mg of AM and 25 mg of PQP were placed in 100 ml volumetric flask and dissolved in 75 ml of Methanol and the volume was made up to the mark

with Methanol, to obtain the solution of 100 µg/ml and 250 µg/ml respectively.

Preparation of working standard solution

Suitable aliquots of above solution were diluted up to the mark with methanol to get the concentration range of 2.5-8.75 for AM and 12.5-43.5 for PQP.

Selection of Detection Wavelength

AM (5 µg /ml) and PQP (25µg/ml) in Methanol, both the solutions were scanned over range of 390-190nm against Methanol as blank, using medium scan speed. The sampling wavelength for analysis, Overlay spectra shows, Absorption maxima (λmax) of AM =276 nm, Absorption maxima (λmax) of PQP =216 nm, Isobestic point = 242nm

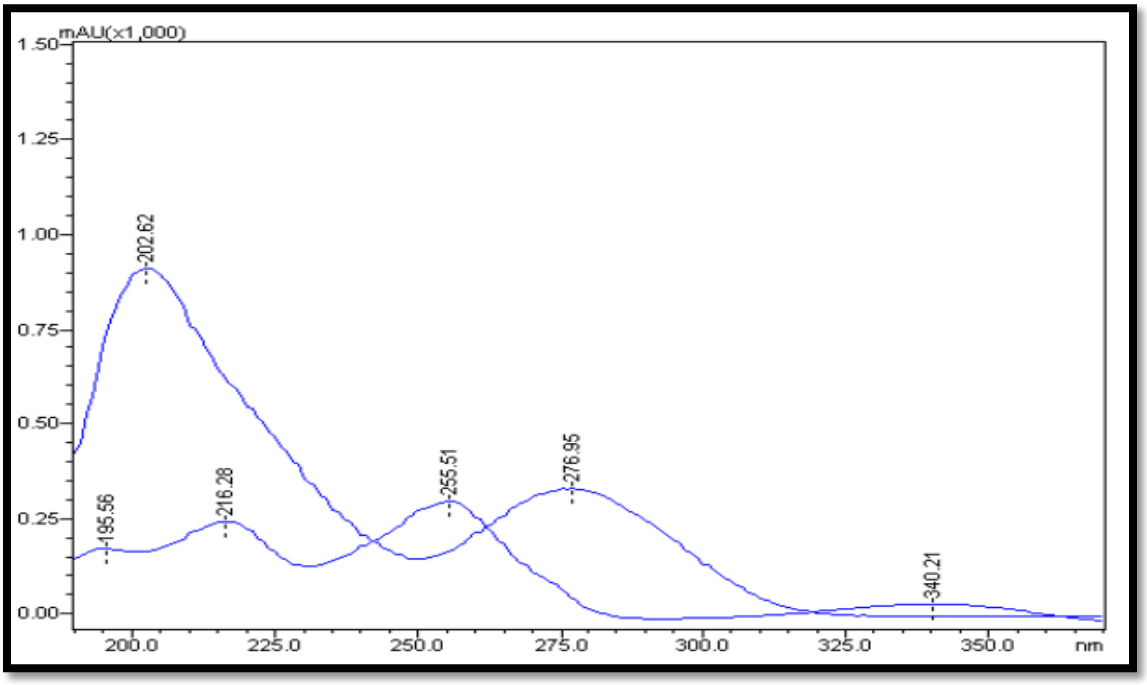

Fig. 3: overlay zero order absorption spectra of standard solutions of AM (2.5-8.75µg/ml) and PQP (12.5-43.75µg/ml) in methanol.

Calibration curve for AM and PQP

To check linearity of the method, working standard solution having concentration in range of 2.5-8.75 µg/ml for AM and 12.5-43.5 µg/ml for PQP were prepared from the standard stock solutions of both drugs. The absorbance was measured at 276 nm (λmax of AM) and at 242 nm (iso-absorptive point). Calibration curves were constructed by plotting concentration vs absorbance.

METHODOLOGY

Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an iso-absorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that AM and PQP show an iso-absorptive point at 242 nm. The second wavelength was selected 276 nm, which is the λ-max of AM. Working standard solutions having concentration 2.5, 3.75, 5, 6.25, 8.75 µg/ml for AM and 12.5, 18.75, 25, 31.25, 43.75 µg/ml PQP were prepared in methanol and the absorbance at 242 nm (iso-absorptive point) and 276 nm (λ-max of AM) were measured and absorptivity coefficients were calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using following equations.

CX = [(QM – QY) / (QX -QY)] × A1/ax1..... (1)

CY = [(QM – QX) / (QY -QX)] × A1/ay1 …… (2)

Where, A1 and A2 are absorbances of mixture at 276 nm and 216 nm; ax1 and ay1
are absorptivities of AM and PQP at 276 nm; ax2 and ay2 are
absorptivities of AM and PQP respectively at 216 nm; QM = A2 / A1,
QX = ax2 / ax1 and QY = ay2 / ay1.

Quantitative estimation of AM and PQP in marketed Tablet Formulation

Formulation

Label claim for content drug is as follow
Arterolane maleate: 150mg
Piperaquine phosphate: 750mg

Twenty tablets were finely powered. A quantity of powder equivalent was weighed and transferred to 100 ml volumetric flask. 60 ml methanol was added to the same flask and sonicated for 15 min. The volume was made up to 100 ml with methanol. The solution was first filtered using Whitman filter paper No.41 and than through 0.45 µ filters paper in order to remove the excipient. After filtration, aliquots solutions were prepared by taking 5 ml sample stock solution. Volume was made up to 100 ml with methanol to produce of 5 µg/ml of AM and 25 µg/ml of PQP.

VALIDATION OF DEVELOPED METHOD

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. Linear correlation was obtained between concentration vs absorbance of AM and PQP. The Linearity spectra and calibration curves of these two drugs at 276 nm and 216 nm are shown in Figure respectively. Calibration curve data of AM and PQP are shown in Table 1 and 2.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, 20 tablets were taken and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80 %, 100 % and 120 %) taking into consideration percentage purity of added bulk drug samples. Results are shown in table 3 and 4.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous sample. It provides an indication of random error in results and was expressed as % RSD.

Intermediate precision (Reproducibility)

Variations of results within same day and amongst days are called as reproducibility. It includes following parameter,

Intra-day reproducibility

A variation of results within same day is called intraday variation. It was determined by repeating calibration curve 3 times on same day. Results are shown in.

Inter-day reproducibility

Variation of results amongst day is called interday variation. It was determined by repeating calibration curve daily for 3 different days. Results are shown in.

RESULTS AND DISCUSSION

Linearity

Table 1: linearity data for AM at 242 nm and 276 nm in methanol

AM			
Concentration (µg/ml)	**Absorbance (242nm)**	**Concentration (µg/ml)**	**Absorbance (276nm)**
2.5	0.262	2.5	0.181
3.75	0.385	3.75	0.265
5	0.532	5	0.375
6.25	0.685	6.25	0.458

8.75	0.882	8.75	0.675
Correlation coefficient: 0.995		Correlation coefficient: 0.998	
Intercept:0.010		Intercept: 0.043	
Slope: 0.019		Slope: 0.052	
Regression Equation: y= 0.019x+0.010		Regression Equation: y=0.052x+0.043	
LOD: 0.432		LOD: 0.657	
LOQ: 1.315		LOQ: 1.992	

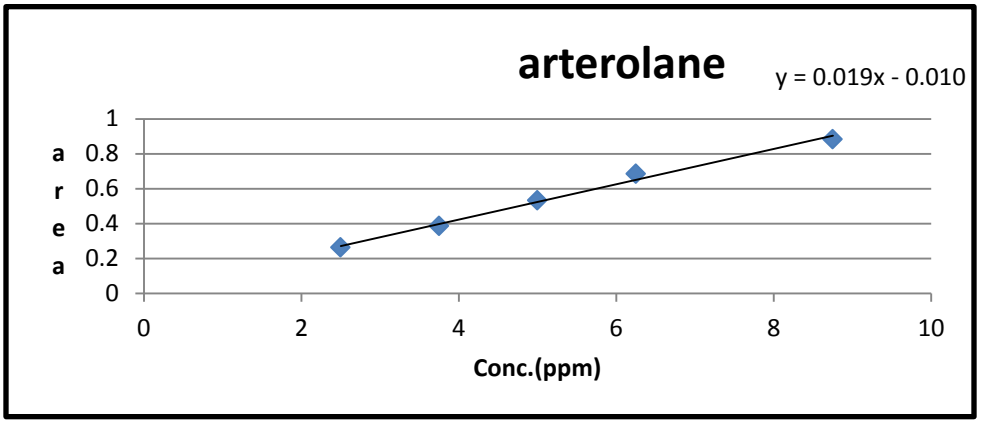

Fig. 4: calibration curve for AM at 242 nm

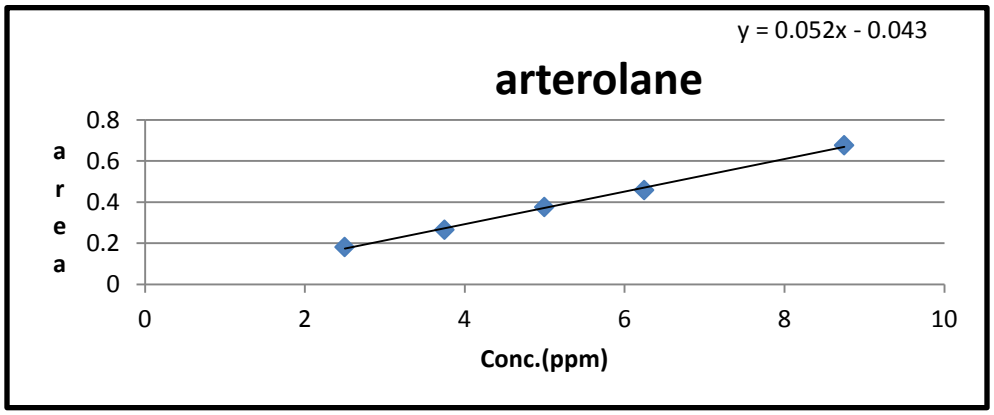

Fig. 5: calibration curve for PQP at 276 nm

Table 2: Linearity data for PQP at 242 nm and 276 nm in methanol

PQP			
Concentration (µg/ml)	**Absorbance (242nm)**	**Concentration (µg/ml)**	**Absorbance (276nm)**
12.5	0.132	12.5	0.06
18.75	0.235	18.75	0.142
25	0.331	25	0.258
31.25	0.395	31.25	0.425
43.75	0.585	43.75	0.574
Correlation coefficient: 0.998		Correlation coefficient: 0.990	
Intercept: 0.106		Intercept: 0.117	

Slope: 0.009	Slope: 0.019
Regression Equation: y= 0.009x+0.106	Regression Equation: y=0.019x+0.117
LOD: 0.403	LOD: 1.111
LOQ: 1.222	LOQ: 3.333

DISCUSSION

AM and PQP were given linear response from 2.5-8.75 µg/ml and 12.5-43.5 µg/ml in Q- Absorbance Ratio method.

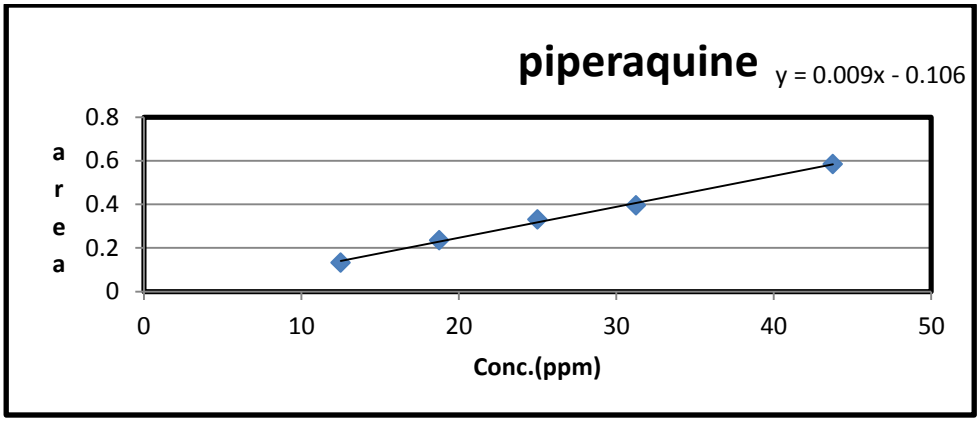

Fig. 6: Calibration curve for PQP at 242 nm

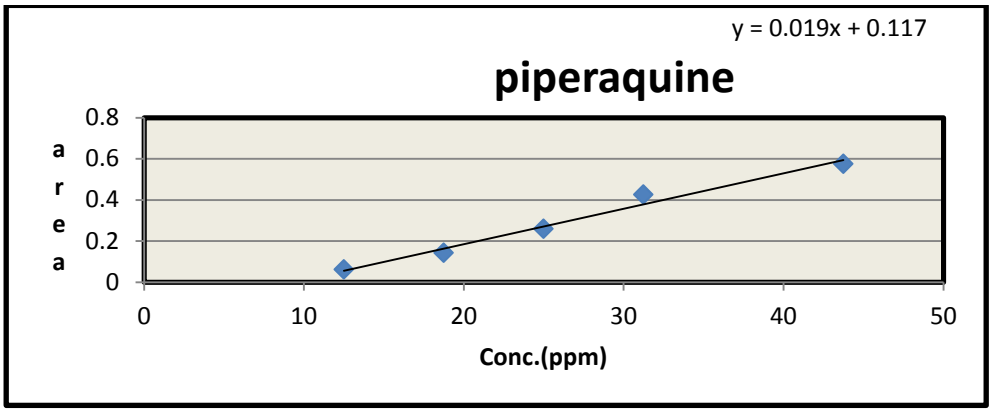

Fig. 7: Calibration curve for PQP at 276 nm

ACCURACY

Table 3: Table recovery data of AM for developed method

% level of recovery	Concentration of Sample Taken (µg/ml)	Concentration of Pure API spiked (µg/ml)	Mean Total Concentration Found* (µg/ml)	%Recovery Mean*	%RSD
80	5	4	3.97	99.42	1.57
100	5	5	5.05	101.22	0.87
120	5	6	5.94	99.36	0.62

***denotes average of three determination**

Table 4: recovery data of PQP for developed method

% level of recovery	Concentration of Sample Taken (µg/ml)	Concentration of Pure API spiked (µg/ml)	Mean Total Concentration Found* (µg/ml)	%Recovery Mean*	%RSD
80	12.5	10	9.97	99.46	1.17
100	12.5	12.5	12.42	99.46	1.08
120	12.5	15	15.11	100.80	1.53

***denotes average of three determination**

DISCUSSION

Result reveals that % recovery of AM and PQP was within acceptance criteria given in ICH i.e. 98-102%

METHOD PRECISION

Intermediate precision (Reproducibility)

The intra-day and inter-day precisions of the developed method was determined by analyzing corresponding responses in triplicate on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of AM (2.5, 5 and 8.75µg/ml) and PQP (12.5, 25 and 43.5 µg/ml). Results were reported in terms of % RSD.

Table 5: Intra-day precision data for AM of 242 nm and 276 nm

Concentration (µg/ml)	Absorbance at 242 nm		Absorbance at 276 nm	
	Mean* ± SD	% RSD	Mean* ± SD	% RSD
2.5	0.184 ± 0.002	1.129	0.194± 0.002	1.03
5	0.366 ± 0.002	0.567	0.359± 0.003	0.83
8.75	0.519 ± 0.004	0.778	0.500± 0.004	0.80

***denotes average of three determination**

Table 6: Intra-day precision data for PQP of 242 nm and 276 nm

Concentration (µg/ml)	Absorbance at 242 nm		Absorbance at 276 nm	
	Mean* ± SD	% RSD	Mean* ± SD	% RSD
12.5	0.133± 0.001	1.145	0.230± 0.002	0.86
25	0.335 ± 0.004	0.190	0.440± 0.003	0.68
43.5	0.582 ± 0.003	0.524	0.726± 0.004	0.55

***denotes average of three determination**

Table 7: Inter-day precision for AM of 242 nm and 276 nm

Concentration (µg/ml)	Absorbance at 242 nm		Absorbance at 276 nm	
	Mean* ± SD	% RSD	Mean* ± SD	% RSD
2.5	0.186 ± 0.001	0.819	0.197± 0.001	0.507
5	0.375 ± 0.003	0.935	0.365± 0.002	0.547
8.75	0.574 ± 0.003	0.611	0.611± 0.003	0.490

***denotes average of three determination**

Table 8: Inter-day precision for PQP of 242 nm and 276 nm

Concentration (µg/ml)	Absorbance at 242 nm		Absorbance at 276 nm	
	Mean* ± SD	% RSD	Mean* ± SD	% RSD
12.5	0.136± 0.002	1.841	0.170± 0.002	1.170
25	0.335 ± 0.004	1.240	0.436± 0.003	0.688
43.5	0.587 ± 0.002	0.354	0.754± 0.001	0.132

***denotes average of three determination**

DISCUSSION

Result reveals that SD and % RSD of AM and PQP was within acceptance criteria given in ICH i.e. less than 1 and less than 2 respectively. So, the proposed method for estimation of AM and PQP in précised in nature.

Quantitation estimation of AM and PQP marketed formulation

The proposed method was evaluate in the assay of table formulation containing AM and PQP. Three replicate determinations were carried out on tablets. % assay found was % for AM and that for PQP was %. Result is shown in table 9.

Table 9: Quantitative estimation of AM and PQP in marketed formulation

Parameters	SYNARIAM TABLET	
	AM	PQP
Actual Concentration (µg/ml)	150	750
Concentration Obtained* (µg/ml)	146.60	739
% Assay*	97.74	98.53
%RSD *	1.690	0.488
Limit	90-110%	90-110%

***denotes average of three determination**

DISCUSSION

% assay of AM and PQP was found in an acceptance limit so this method could be used for analysis of this combination.

CONCLUSION

The described method enables the quantification of AM and PQP in combined tablet dosage form. The validation data demonstrates good precision and accuracy, which prove the reliability of proposed method. This method was based on the determination of graphical absorbance at two wavelengths, one being Iso-absorptive point for the two drugs (242 nm) and the other being the wavelength of AM (276nm).

Hence, this Q- Absorbance Ratio method can be used routinely for quantitative estimation of both components in solid dosage form.

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