Method development and validation for the simultaneous estimation of Arterolane maleate and Piperaquine phosphate by HPLC in pharmaceutical dosage form (Tablet)

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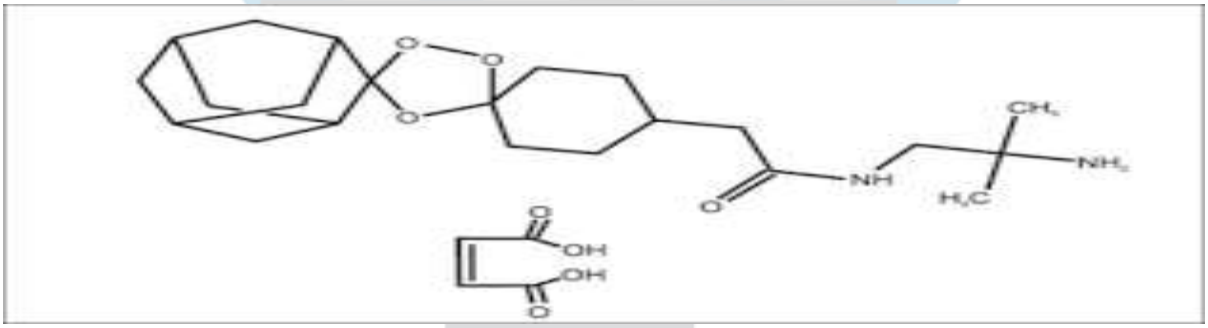
***Abstract*: A novel, precise, accurate, rapid and cost effective gradient high performance liquid chromatographic (HPLC) method was developed, optimized and validated for the estimation of Arterolane maleate and Piperaquine phosphate in pharmaceutical dosage forms (tablet). The drugs were estimated using JASCO C-18HS (250 mm x 4.6 mm i.d- 5 µm particle size) column. A mobile phase composed of phosphate buffer, methanol in proportion of 70:30 v/v, at a flow rate of 1.0 ml/min was used for the separation. Detection was carried out at 270 nm. The linearity range obtained was 5-30 µg/ml for ART and 10-60 µg/ml for PIP with retention times (RT) of 3.735 min and 2. 839 min for ART and PIP respectively. The correlation coefficient values were found to be 0.999. Precession studies showed % RSD values less than 2 % for both the drugs in all the selected concentrations. The percentage recoveries of ART and PIP were in the range of 99.64-100.28% and 99.84-100.78% respectively. The assay results of ART and PIP were 99.63% and 99.81 % respectively. The limit of detection (LOD) and limit of quantification (LOQ) were 0.168 µg/ml 0.875 µg/ml for ART and 1.632 µg/ml and 2.741 µg/ml for PIP respectively. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available dosage form.**

***Keywords*: Arterolane maleate, Piperaquine phosphate, HPLC, Validation**

Introduction

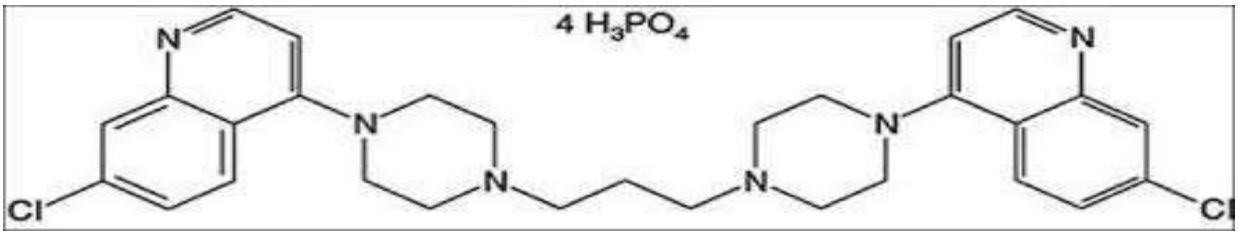
Arterolane maleate (AM) is chemically known as [(N-(2-amino-2-methylpropyl)-2-cis-dispiro (admantane-2, 3'-[1, 2, 4] trioxolane-5, 1"-cyclohexan)-4"-yl] acetamide maleate. Arterolane maleate is synthetic peroxide which acts as anti-malarial agent by rapid acting as blood schizonticides against all blood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

Figure 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]

Figure 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. Central

Drug Standard Control Organization (CDSCO) approved the combination of AM and PQP on dated 19/10/2011.[5] Very few methods like HPLC,[6-8] Capillary zone electrophoresis, [9] LC-MS[10] have been reported as a single or in combination with other drugs. The aim of this proposed work was to develop and validated to simple, rapid, precise, robust and specific method as per International Conference on Harmonization (ICH) guidelines.

Materials and Methods

Instrumentation

Separation and estimation was carried out using HPLC system a JASCO C18 column (250 × 4.6 mm i.d., 5µm particle size) was used. Samples were injected using Rheodyne injector with 20 µL loop and detection was carried out using UV detector. Data was analyzed by using Spinchrom software.

Chemicals and solvents

Arterolane maleate and piperaquine phosphate were procured from KP labs, Hyderabad, India as a gift sample. HPLC grade solvents: Water, methanol were obtained from Merck India Ltd., Mumbai. SYNRIAM Tablet (Arterolane maleate 150 mg and Piperaquine phosphate 750 mg) was procured from local market.

Chromatographic conditions

The mobile phase consisted of phosphate buffer (pH 6.0, adjusted with orthophosphoric acid) and Methanol in a proportion of 70:30 v/v. The flow rate was 1 ml/min. Although the Arterolane maleate and Piperaquine phosphate have different wavelength, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 270 nm was selected as a detection wavelength.

Preparation of mobile phase

Mobile phase was prepared by mixing 700 ml phosphate buffer (pH 6) and 300 ml Methanol. Above mixture (70:30) was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Selection of mobile phase for method optimization

Several trials have been taken for the proper optimization of HPLC method by changing different mobile phase with different ratio. And finally the mobile phase for optimized condition phosphate buffer (pH 6): Methanol in the ratio of (70:30 v/v) was selected and chromatogram is shown in Fig.4.

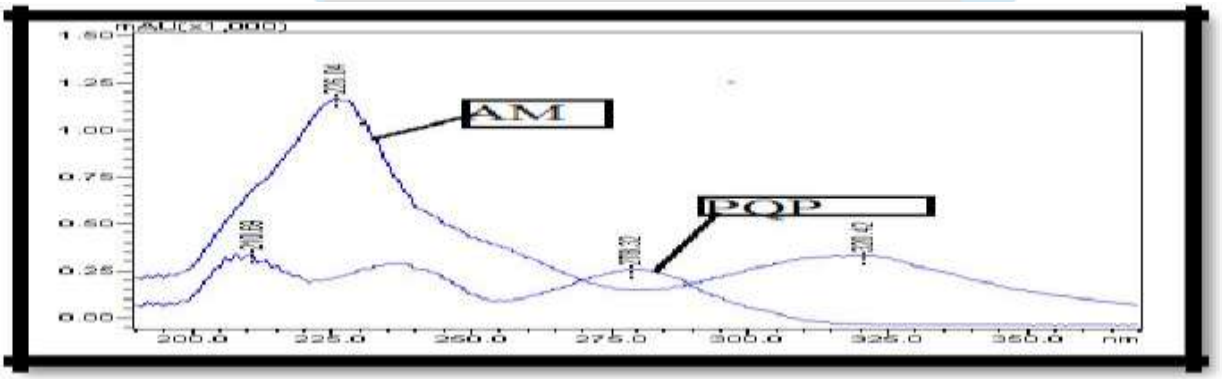


Figure 3: Overlay spectra of AM and PQP.

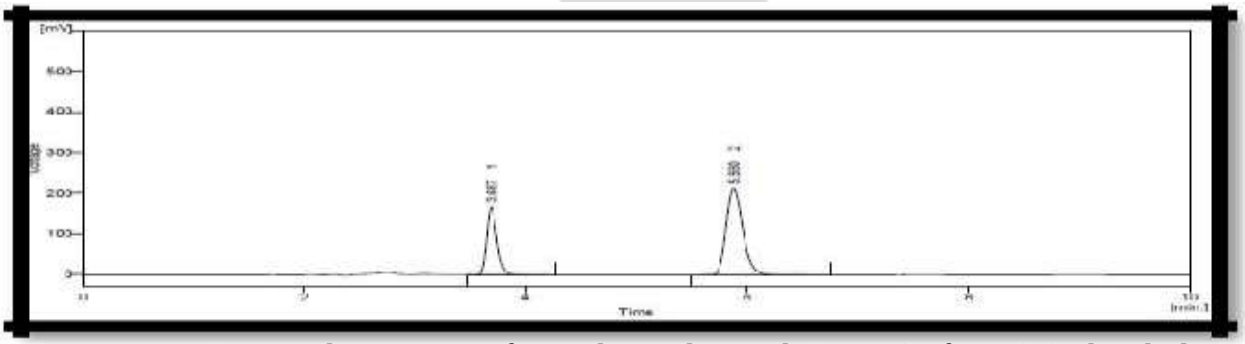


Figure 4: Chromatogram of Arterolane maleate and Piperaquine for optimized method.

Preparation of Standard Solution
Accurately weighed 10 mg of standard AM and 30 mg of standard PQP and transferred to a 100 ml volumetric flask and dissolved in Methanol and sonicated for 15 minutes and volume was made up with Methanol. From these solutions, pipette out 1ml in to 10 ml volumetric flask respectively, and dilute it with Methanol up to the mark to give a solution containing 10 µg/ml AM and 30 µg/ml PQP.

Preparation of sample solution

Twenty tablets were accurately weighed and ground to fine powder. Weigh and transferred tablet powder equivalent to AM 10 mg and PQP 30 mg were transferred into 100 ml volumetric flask containing 100 ml Methanol, sonicated for 30 min and diluted to mark with same solvent and filtered. From the above solution 1 ml was transferred into 10 ml volumetric flask and diluted to mark with same solvent.

Method validation

Linearity and range

By appropriate aliquots of the standard Arterolane maleate and Piperaquine phosphate solutions with the mobile phase, six working solutions ranging between 5-30 µg/ml and 10-60 µg/ml for Arterolane maleate and Piperaquine phosphate respectively. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of both drugs to obtain the calibration curve.

Accuracy

Accuracy of the method was determined by recovery experiments at spiked levels of 50%, 100% and 150%. The samples were prepared in triplicate for each level; the % recovery.

Precision

Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of Arterolane maleate and Piperaquine phosphate. Determinations were performed on the same day as well as on consequent day and % RSD was calculated.

Limit of detection and limit of quantitation

Limit of Detection (LOD) and Limit of Quantification (LOQ) for both drugs were determined by calibration curve method. Solutions of both drugs were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were estimated using the formula:

LOD= 3.3 σ/S and
LOQ = 10 σ/S

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of pH and ratio of mobile phase and flow rate.

Results and Discussion

The results of method development and validation study on simultaneous estimation of AM and PQP in the current study involving phosphate buffer (pH-6): Methanol (70:30 v/v) as the mobile phase for HPLC is given below.

Linearity

The drug response was linear (R^2 = 0.9994 for AM and 0.9998 for PQP) over the concentration range between 5-30 µg/ml for AM and 10-60 µg/ml for PQP.

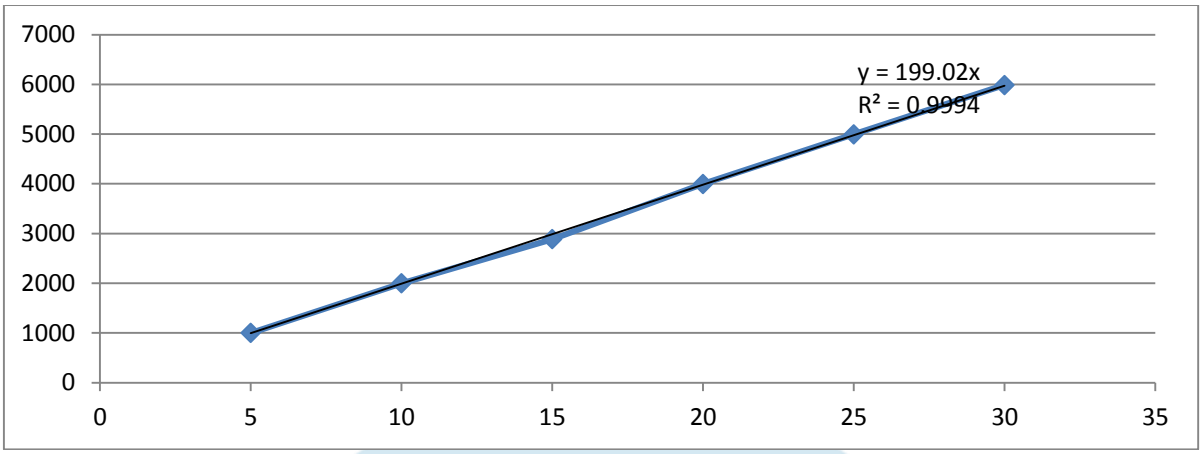


Figure 5: Calibration curve of Arterolane maleate at 270 nm.

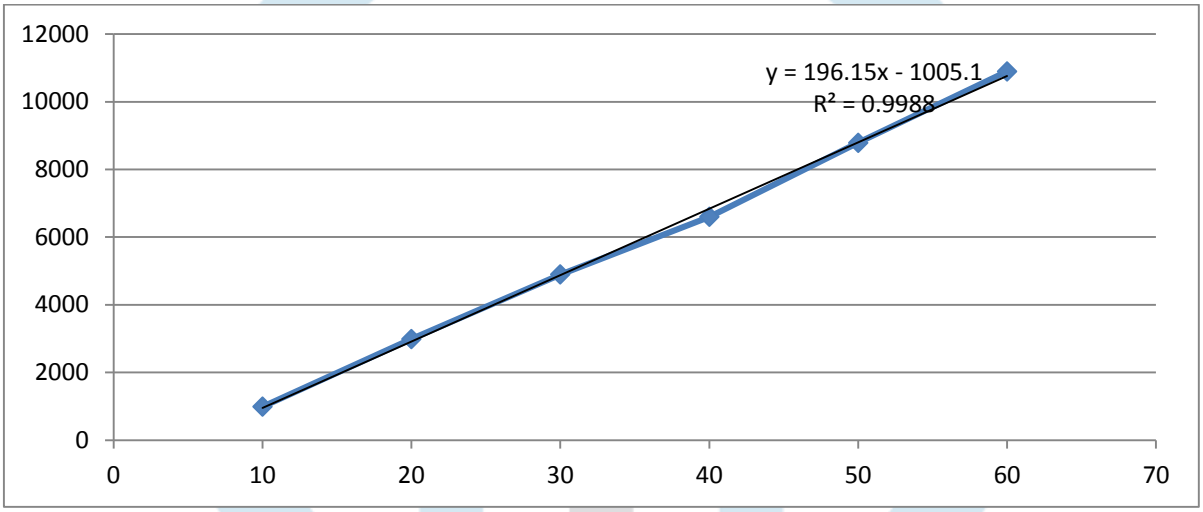


Figure 6: Calibration curve of Piperaquine phosphate at 270 nm.

Accuracy

The recoveries of the both drugs in the range from 98% to 102 % were obtained at various added concentrations.

Precision

The developed method was found to be precise as the RSD values for repeatability of intra-day and inter-day precision studies were < 2%, respectively which is under limit as per recommendations of ICH guidelines.

Robustness

The standard deviation of the peak areas was calculated for each parameter and the %RSD was found to be less than 2%.

System suitability parameters

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and the results are expressed in Table-6. The values obtained demonstrated the suitability of the system for analysis of this drug combination.

Sensitivity

The LOD and LOQ were separately determined based on the calibration curves for AM and PQP. The LOD and LOQ were found to be 0.168 µg/ml 0.875 µg/ml for ART and 1.632 µg/ml and 2.741 µg/ml for PIP respectively.

Table 1: Determination of accuracy for AM.

% Level of Recovery	Conc. Of sample solution (µg/ml)	Conc. Of standard Solution (µg/ml)	Total conc. (µg/ml)	Peak area	Conc. found (µg/ml)	% Recovery
50	5	5	10	525.575	4.99	99.68
100	5	10	15	658.132	4.73	99.73
150	5	5	10	720.901	4.58	99.64

Table 2: Determination of Accuracy for PQP.

% Level of Recovery	Conc. of sample solution (µg/ml)	Conc. Of standard Solution (µg/ml)	Total conc. (µg/ml)	Peak area	Conc.Found (µg/ml)	% Recovery
50	10	15	25	783.86	14.97	99.83
100	10	20	30	1116.60	19.43	99.94
150	10	25	35	1526.09	23.61	99.63

Table 3: Repeatability data of AM and PQP.

Concentration	AM (10 µg/ml)	PQP (30 µg/ml)
Area (NMT-2%)	1098.624	2252.810
	1088.166	2236.104
	1101.041	2223.517
	1110.270	2237.169
	1088.730	2230.052
	1102.989	2231.423
Mean	1098.303	2235.180
± SD	8.569346	9.920320
%RSD	0.78629236	0.447352

Table 4: Intra-day and Inter-day precision of AM and PQP.

Drug	Concentration (µg/ml)	Intra-day Area mean (n=3) ± SD	%RSD	Inter-day area mean (n=3) ± SD	%RSD
	5	564.037±3.769	0.6720323	543.72±3.099	0.5723
	15	1094.015±1.475	0.1343093	1094.10±4. 734	0.4308
AM	20	1646.015±5.551	0.3354900	1590.10±11.398	0.5859
	20	1093.370±3.648	0.3387535	1092.54±13.228	0.3407
	40	2222.516±13.897	0.6883396	2122.05±14.045	0.8153
PQP	50	3233.95±31.454	0.9262924	3522.88±31.431	0.9357

Table 5: Robustness data of AM and PQP.

Parameters	Normal Condition	Change in condition	Drug	Conc (µg/ml)	Mean Area (n=3) ±SD	% RSD	Retention on time (mins)	Theoretical plate
Mobile phase ratio Phosphate buffer: methanol (70:30w/v) **±0.2**	70:30	68:32	**AM**	10	1126.16± 2.583	0.2359	3.45	7011
			PQP	30	2275.49± 5.957	0.261	7.07	7154
		72:28	**AM**	10	1089.98± 11.085	1.064	4.87	7090
			PQP	30	2156.42± 13.160	0.640	5.65	7531
Change in Flow Rate (**±0.2**)	1.0ml/min	0.8 ml/min	**AM**	10	1133.36± 9.425	0.823	3.61	6853
			PQP	30	2321.12± 21.45	0.830	7.15	7207
		1.2 ml/min	**AM**	10	1165.56± 13.48	1.071	4.16	7011
			PQP	30	22571.58 ±14.30	1.043	4.32	7632
Change in P (**±0.2**)	6.0	5.8	**AM**	10	1046.11± 6.19	0.968	3.43	6821
			PQP	30	2482.92± 23.78	1.779	6.09	7021
		6.2	**AM**	10	1130.39± 15.90	1.870	3.54	7104
			PQP	30	2219.98± 20.64	1.129	6.92	7192

Table 6: System suitability parameters of AM and PQP.

Parameters	Data obtained		Specifications
	AM	PQP	
Retention time (Rt)	3.735	2. 839	-
Resolution (Rs)	9.421	9.781	More than 1.5
Theoretical Plates(N)	7011	7156	More than 2000
Tailing factor (Tf)	1.438	1.641	NMT 2

Table 7: Quantitative estimation of pharmaceutical formulation.

Parameters	**Synariam Tablet**	
	AM	**PQP**
Actual Concentration (µg/ml)	150	750
Concentration Obtained (µg/ml)	147.84	742.78
% Assay	98.560120	99.037333
%RSD	0.9967	0.810045
Limit	90-110%	90-110%

Specificity

No interference peaks were found in the chromatogram by proposed HPLC method

Quantitative estimation of pharmaceutical formulation

Experimental results of the amount of AM and PQP in mixture, expressed as a percentage of label claims were in good agreement, thereby suggesting that there is no interference from any of the excipient which are normally present in tablets. In the replicate analysis (n=3) of AM and PQP by proposed method showed that the content of AM and PQP was 99.78% and 100.086% respectively. The retention times of AM and PQP was found to be 3.735 min and 2. 839 min respectively and the resulst of the analysis of tablet are given in Table 7.

Conclusion

From all results, it is concluded that the developed HPLC method is simple, accurate, precise, rapid, selective and robust, thus can be used for routine analysis of Arterolane maleate and Piperaquine phosphate in combined tablet dosage form.

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