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Stability indicating RP-HPLC method development for simultaneous determination of arterolane maleate and piperaquine phosphate in bulk and tablet dosage form

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ABSTRACT

A simple, accurate, precise and stability indicating HPLC method has been developed and validated for simultaneous determination of Arterolane Maleate and Piperaquine Phosphate in pure and tablet dosage forms. Reversed-phase chromatography was performed on C18 column (Thermo Hypersil 100nm ×4.6× nm× 5µm) with methanol – potassium di hydrogen orthophosphate buffer (50:50v/v),as mobile phase flows at flow rate of 1.0ml/min. Detection was performed at 290 nm. The method was validated with respect to linearity, accuracy, precision and robustness. The degradation behavior of drug was investigated under various stress conditions recommended by International Conference of Harmonisation (ICH) guidelines. The retention times of Arterolane and Piperaquine were 1.32 & 3.42 respectively. The linearity range of Arterolane and Piperaquine 50-150 µg/ml with correlation coefficient of 1.The means recovery study was carried by standard method and results were in range of 101 to 100% for Arterolane and 99.9 to 100.0 % for Piperaquine. The relative standard deviation for method precision studies of Arterolane and Piperaquine was 0.9 & 0.51 respectively and for intraday precision 0.30 & 0.19. The average % assay was 99% for both Arterolane and Piperaquine. There was no interference of peak obtained for degraded product with sample peaks which ensures the specificity of the method. Statistical analysis proved the method was precise, reproducible, selective, specific and accurate for analysis. The wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase imply the method is suitable for routine quantification of Arterolane and Piperaquine with high precision and accuracy.

Key words: Arterolane, Piperaquine, HPLC, validation, Forced degradation.

INTRODUCTION

Arterolane (RBX 11160) Maleate is a new, fully synthetic 1, 2, 4-trioxolane with a peroxidic pharmacophore and is a rapidly acting oral antimalarial drug [1]. Arterolane Maleate chemically described as cis-adamantane-2-spiro-3-8-[[[(2-amino-2 methylpropyl) amino] carbonyl] methyl] 1, 2, 4-trioxaspiro [4.5] decane hydrogen Maleate. Fig 1.Its empirical formula is $C_{26}H_{40}N_2O_8$ and molecular weight is 508.61 [2] Arterolane is more active than artemisinin and is cheap to produce. It has a longer lifetime in plasma, so it stays active longer in the body [3]. Arterolane shows high potency in vitro than that of chloroquine, mefloquine, artemether, and artesunate. In vivo, Arterolane is highly effective against plasmodium berghei in mice [4]. Arterolane causes lipid peroxidation, damages endoplasmic reticulum, inhibits protein synthesis and ultimately results in lysis of the parasite [5].

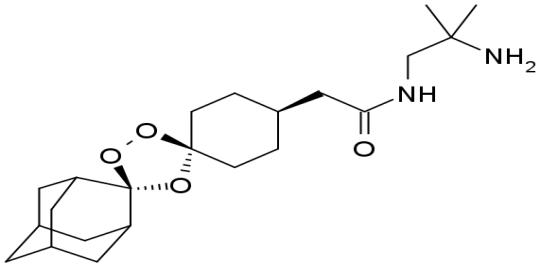

Fig 1: Structure of Arterolane Maleate

Piperaquine phosphate is an Antimalarial compound belonging to the 4-aminoquinolines compound [6]. The chemical name of Piperaquine is 1,3-bis[4-(7-chloroquinolyl-4)-piperazinyl-1]propane tetraphosphate tetrahydrate 2,3. Fig 2. The molecular formula is $C_{29}H_{32}C_{12}N_6$**.** $4H_3PO_4$**.** $4H_20$ and molecular weight is 999.56 [7]. It is a highly liphophilic. Piperaquine interferes with the degradation of haemoglobin by parasitic lysosomes, helps in damaging of plasmodial membranes [8]. It has no marked effect on the ring forms, immature or mature schizonts and the male of female gametocytes [6].

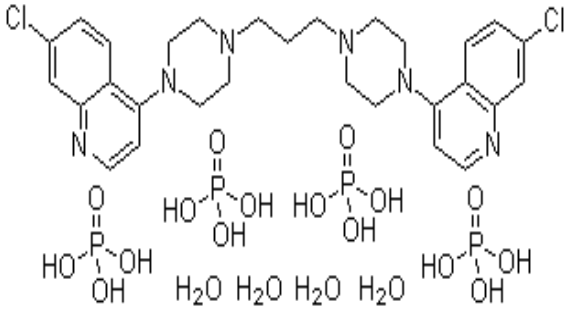

Fig 2: Structure of Piperaquine Phosphate

Literature survey reveals that, there are few, HPLC [7-8], LCMS [9], Capillary Zone electrophoresis. Methods are reported for the simultaneous analysis of Arterolane and Piperaquine in bulk and tablet dosage forms.

So that need was felt, to develop new simple, economical, precise and stability indicating high performance liquid chromatographic (HPLC) method with a wide linear range and good sensitivity for assay of Arterolane and Piperaquine in bulk and tablet dosage forms using PDA detection.

The method was validated according to standard International Conference on Harmonization (ICH) guidelines [10] and various experimental parameters were optimized

MATERIALS AND METHODS

Reagents:
Methanol (HPLC grade), Water (HPLC grade), HCl, H2O2, NaOH.

Chemicals:
Pure samples of Arterolane and Piperaquine

Synriam tablets (150 mg of Arterolane Maleate and 750 mg of Piperaquine Phosphate)

Buffer (Potassium di hydrogen ortho Phosphate)

Instrument and Chromatographic Conditions:
The liquid Chromatographic study was performed on Water's system provided with syringe, auto sampler, 2996 PDA detector and reporting were performed by Empower 2 software.

Chromatographic analysis was performed using Thermo Hypersil BDS C18 (100nm × 4.6nm × 5µm) using Sartorious – Digital weighing balance. The mobile phase in the ratio of 50:50 v/v, which was degassed before use The PH was adjusted to 4.5 with Ortho Phosphoric Acid OPA). The chromatography performed at a flow rate of 1.0 ml/min using buffer and methanol in 50:50 ratio. The column temperature was ambient and the Detection wave length was at 290 nm. The Injection volume was 1µl and the run time was 1.32 min for Arterolane and 3.42 min for Piperaquine.

Preparation of Solutions:
Standard solution:
15 mg of Arterolane Maleate and 75 mg of Piperaquine Phosphate working standard were taken in 100ml volumetric flask. It was dissolved in 10ml water and made up to the mark with the water. It was degassed in ultra sonicator and then filtered through membrane filter of 0.45µ pore size.

Sample solution:
10 tablets were crushed and powder equivalent to 1340 mg was taken into 100ml volumetric flask. It was made to dissolve with water and made up to the mark with water. The solution was degassed and filtered through membrane filter paper of pore size 0.45µ.

Transfer the above 5ml solution into 50 ml volumetric flask and make up the volume with water.

Buffer solution:
13.609 gm of Potassium di hydrogen Phosphate was dissolved in 1000ml of water. The solution was adjusted to a PH of 4.5 with Ortho Phosphoric acid (OPA). Then it was degassed in ultrasonicator for 10 min and then filtered through 0.45 µ pore size membrane filter paper.

Mobile Phase:
Mix a mixture of 500ml buffer and 500 ml of Methanol HPLC grade and degas in ultra sonic water bath for 10 min. Filter through 0.45 µ filter under vacuum filtration.

Degradation Solutions:
Acid Stress:
1340 mg of sample was taken and transferred to 100 ml of flask to this 10ml of 0.1 HCL solution is added and refluxed for 30 min at 60^0C and cool to room temperature and add 10 ml of 0.1N NaOH to neutralize and make up to mark with water.

Base Stress:
1340 mg of sample was taken and transferred to 100 ml of flask to this 10ml of 0.1N NaOH solution is added and refluxed for 30 min at 60^0C and cool to room temperature and add 10 ml of 0.1N HCL to neutralize and make up to mark with water.

Oxide Stress:
1340 mg of sample was taken and transferred to 100 ml of flask to this 10 ml of 1 % H_20_2 and refluxed for 30 min at 60^0C and cool to room temperature and make up to mark with water.

Hydrolytic stress:
1340 mg of sample was taken and transferred to 100 ml of flask and add 10 ml of H_2O and refluxed for 30 min at 60^0C and cool to room temperature and make up to mark with water.

Thermal stress:
1340 mg of sample was taken and exposed to 105^0C for 6 hrs and transferred to 100 ml of flask and add refluxed for 30 min at 60^0C and cool to room temperature and make up to mark with water.

Method Development:
After several trails with various solvents, mobile phase system composed of methanol and buffer in the proportion of 50:50 was chosen for the simultaneous estimation of Arterolane and Piperaquine in combined dosage form by RP-HPLC. This mobile Phase composition offered maximum resolution for the drug at the detection wavelength 290 nm.

Mobile phase with the flow rate of 1 ml/min gave optimum separation with good resolution between the peaks. A reverse phase C18 column was used as stationary phase. The retention time of Arterolane and Piperaquine were found to be 1.3 and 3.4 min, respectively. The total time of analysis was less than 5 min. The % Recovery for Arterolane and Piperaquine were found to be 100 and 99 % respectively.

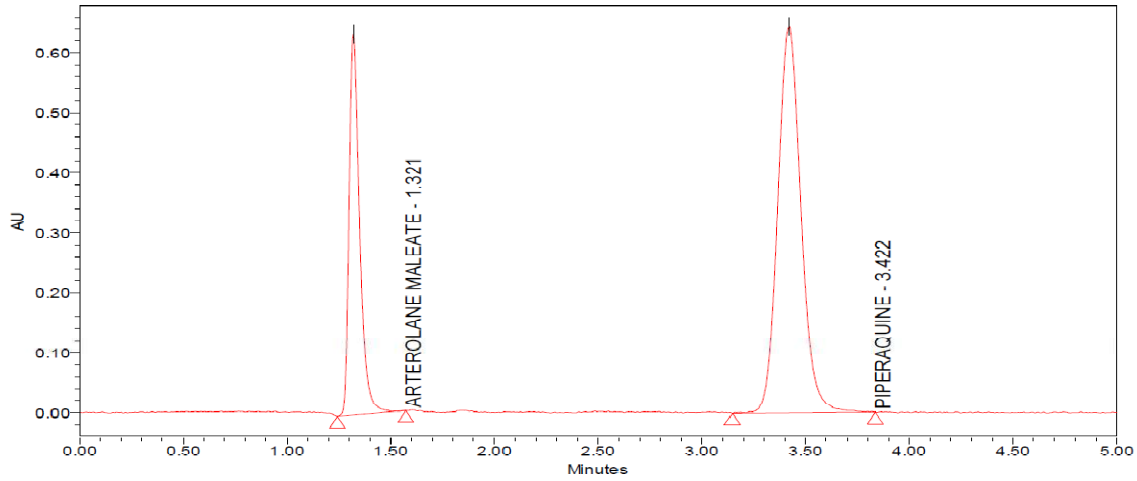

Fig 3: HPLC Chromatogram of Standard Arterolane and Piperaquine

METHOD VALIDATION:
System suitability:
System suitability was studied to ensure the validity of the analytical procedure by injecting six replicates of the standard solution. That is used for the evaluation of the system suitability parameters like theoretical plates per meter, tailing factor, Percentage relative standard deviation of area and retention time of these six injections. Analysis was carried out and the values are well within the limits.

Specificity:
Mobile phase along with placebo were injected to check the interference at the RT of analytes in above mentioned chromatographic conditions. By comparing the chromatograms of blank, standard and sample, it was found that there is no interference due to excipients and also found good correlation between the retention times of standard and sample.

Linearity:
The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in sample. Appropriate aliquots of Arterolane and Piperaquine solutions were within concentration, ranging between 50-150 µg/ml from the standard stock solution and filtered through a 0.4 µm membrane filter. The experiment was performed in according to optimized chromatographic conditions. The linearity was evaluated by linear regression method.

Accuracy:
Accuracy of the method was calculated by performing recovery studies. The recovery experiments were performed by adding known amounts of the drug in the placebo. It is carried out by preparing the samples at concentration levels of 50 %, 100 %, and 150% of test concentration. Recoveries (%), RSD (%) were calculated for each concentration.

Precision:
Precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogeneous sample. It was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intraday and interday precisions were determined by analyzing the samples of Arterolane and Piperaquine. Six replicates concentration of the working sample solution were analyzed to calculate statically valid estimate of % Relative Standard Deviation (% RSD).

LOD & LOQ:
Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ respectively. To determine the LOD and LOQ serial dilutions of mixed standard solution of Arterolane and Piperaquine was made from the standard stock solution. The samples were injected in HPLC system and average peak area was plotted against concentration. LOD and LOQ were calculated.

Robustness:
The ability of a method to remain un affected by small and deliberate variations in method parameters provide an indication of its reliability for routine analysis and this can be evaluated by performing robustness studies. It was performed by altering the experimental conditions like column temperature, flow rate, buffer ph, and mobile phase composition without change in system suitability parameters. The flow rate of the mobile phase was changed by 20% to 0.8 ml/min and 1.2 ml/min and the column temperature was also changed by ± 5^0C and the effect was studied.

RESULTS AND DISCUSSION

System suitability:
All System suitability parameters like theoretical plates per meter, peak tailing, and similarity factor, percentage relative standard deviation of area and retention time of six injections were carried out and results (table 1) were found to be within the limits.

Table 1: System suitability parameters

Parameters	Arterolane	Piperaquine
Theoretical plates	3398	4463
Similarity factor	1	1
Retention time	1.3	3.4
Tailing	1.4	1.1
% RSD	0.5	0.4

Linearity:
Arterolane and Piperaquine show linearity response between 50-150 µg/ml with good correlation coefficients (r^2=1 for both drugs). The area of each peak was plotted against the concentration to obtain the calibration graph (Fig.4 & Fig.5). The values of the slope and intercept were y=22571x-861.9 for Arterolane and y=50594x+11778 for Piperaquine and results were shown in table 2-3.

Table 2: Linearity data for Arterolane

Linearity level	Concentration(µg/ml)	Area
1	50	1122930
2	75	1698355
3	100	2257401
4	125	2812585
5	150	3388866
6	Correlation coefficients	1

Table 3: Linearity data for Piperaquine

Linearity level	Concentration(µg/ml)	Area
1	50	2554752
2	75	3815023
3	100	5065719
4	125	6338375
5	150	7593760
6	Correlation coefficients	1

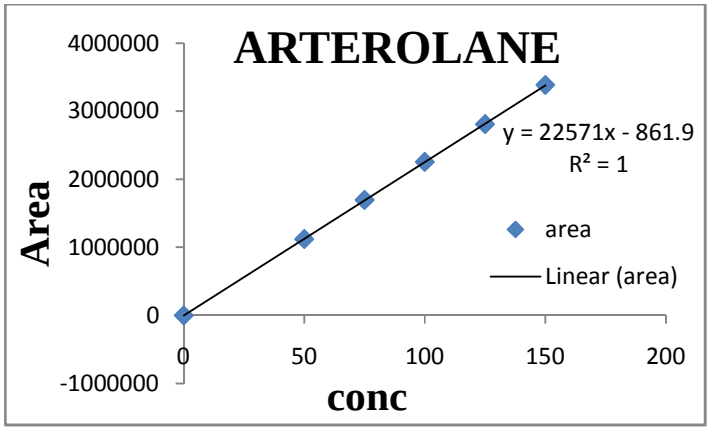

Fig 4: Calibration Curve of Arterolane

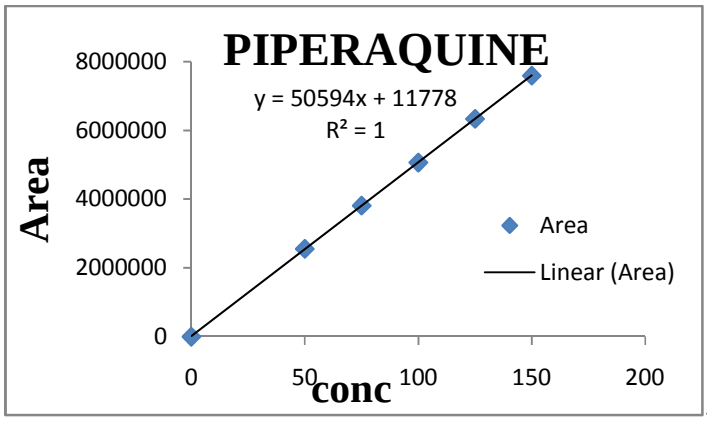

Fig 5: Calibration Curve of Piperaquine

Accuracy:

Good recoveries results were obtained within the acceptance limits, which is indicative of high accuracy. The recovery was performed at three levels, 50%, 100% and 150%. The solutions were prepared in triplicates and the accuracy was indicated by % recovery. The recovery values for Arterolane and Piperaquine ranged from 101 to 100% and 99.9 to 100.0 % respectively. The results were shown in table 4-5.

Table 4: Accuracy results of Arterolane Maleate

Spiked level	Sample area	Amount added	Amount found	% Recovery
50%	1138569	74.324	75.17	101
100%	2259305	148.538	149.16	100
150%	3380131	223.083	223.65	100

Table 5: Accuracy results of Piperaquine Phosphate

Spiked level	Sample area	Amount added	Amount found	%Recovery
50%	2521397	371.621	370.33	100
100%	5069625	742.688	744.60	99.9
150%	7596052	1115.417	1115.67	100

Precision:

The results of intraday and method precision as shown in table 6 revels that the method developed is highly precise since the %RSD values are extremely low (limit < 2%) i.e. for method precision studies of Arterolane and Piperaquine was 0.9 & 0.5 respectively and for intraday precision 0.3 & 0.19.

Table 6: Method and Intraday precision results of Arterolane and Piperaquine

Sample no/parameters	Method precision(%Assay)		Intraday(%Assay)	
	Arterolane	Piperaquine	Arterolane	Piperaquine
Sample 1	97	99	99	99
Sample 2	100	100	100	99
Sample 3	99	99	99	99
Sample 4	98	98	99	99
Sample 5	99	99	100	99
Sample 6	99	99	99	99
Avg Assay	99	99	99	99
STD	0.89	0.51	0.30	0.19
% RSD	0.90	0.51	0.30	0.19

LOD & LOQ:

LOD and LOQ for Arterolane were 0.22 and 0.75 µg/ml respectively and for Piperaquine were 0.11 and 0.37 µg/ml respectively.

Robustness:

The robustness of the developed method is revealed by the low % RSD obtained in the readings between runs done employing the optimized chromatographic conditions and that employing the deliberately altered conditions as shown in table 7.

Table 7: Robustness results of Arterolane and Piperaquine

	Flow rate		Temperature	
	0.8ml/min	1.2ml/min	25^0C	30^0C
Arterolane Maleate	1.64	1.10	1.33	1.32
Piperaquine Phosphate	4.26	2.84	3.55	3.41

Assay of marked formulation:

Ten tablets were weighed and grounded to a fine powder. An amount of powder equivalent to 150 mg of Arterolane and 750 mg of Piperaquine were weighed accurately and transferred into 100 ml volumetric flask containing 25 ml of water and sonicate for 20 min and make up to the mark with water, then the solution was filtered through 0.45µm membrane filter and 5ml of filtrate take into 50ml of volumetric flask and made upto the volume with water. Chromatograms were recorded at 290 nm. Content of drug in sample solution was calculated by comparing mean peak area of sample with that of the standard. The results were shown in table 8.

Table 8: Assay results of Arterolane and Piperaquine

Drug	Label Claim	Amount found	Assay
Arterolane Maleate	150	149.16	99.44
Piperaquine Phosphate	750	744.60	99.28

Forced degradation studies:

Suitable aliquots from each stressed sample were prepared and injected to HPLC system under optimized chromatographic conditions and the chromatograms were observed for interferences and the results were shown in the table9.

Table 9: Degradation results of Arterolane and Piperaquine

Condition	% deg of Arterolane	% deg of Piperaquine
Acid	-5	-3
Base	-10	-5
Peroxide	-8	-3
Heat	-3	-6
Light	-14	-8

CONCLUSION

A validated stability indicating assay method has been developed for the determination of Arterolane and Piperaquine in bulk and in tablet dosage form. A new mobile phase was found during the method development process. The results show that the developed method was accurate, precise, simple, economic, fast, specific, and linear and found to be stability indicating under stress conditions.

REFERENCES

[1] Vennerstrom JL, Arbes-Barnes S, Brun R. *Nature* **2004**; 430:900-4.
[2] http://www.synriam.com/medical-professionals.
[3] Abishek Gupta, Yogendra S, Kona S, Garima J. *Journal of Pharmacy and BioAllied Sciences* 2**0**10; 2(1):32-37.
[4] Neena V, Sornchai L, Andreas M. *Clinical infectious disease* **2010**; 51(6):684-691.
[5] Tripathi KD, **2008**. Essentials of Medical Pharmacology. 6th ed. Jaypee Brothers Medical Publishers; Page No: 708-98.
[6] http://druginfosys.com/drug.aspx?drugcode=2227&drugname=piperaquine%20phosphate&type=0
[7] Venkata raveendra babu V, Pankaj kumar S, Indrajeet S. *International Journal of Research in Pharmacy and Chemistry* **2013**; 3(3):718-23.
[8] Swathi I, Nayeem N, Sandeep K. *International journal of universal Pharmacy and Bio sciences* **2013**; 2(4):424-431.
[9] Lindegardh N, Annerberg A, White NJ, Day NJP. *Journal of Chromatography* **2008**; 1(2): 227-236.
[10] ICH harmonised triparitite guidelines validation of Analytical procedures: Text and Methodology Q2 (R1) Nov **2005**.