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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ARTEROLANE MALEATE AND PIPIERAQUINE PHOSPHATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

The drug of Arterolane maleate and Piperaquine phosphate were injected into the HPLC system and run in different solvent systems. From the overlaid spectra, 228 nm was selected as analytical wavelength for multi component analysis using HPLC method. The marketed solution was analyzed for the estimation of drug by proposed method. The system suitability parameters were evaluated from standard Chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from five replicate injections.Assay was performed in triplicate for various concentrations of Arterolane maleate and Piperaquine phosphate equivalent to 50, 100 and 150 % of the levels was injected into the HPLC system per the test procedure. The average % recovery of both Arterolane maleate and Piperaquine phosphate was calculated and the results were summarized. The accuracy studies were shown as % recovery for Arterolane maleate and Piperaquine phosphate at 50%, 100% and 150% the limits of % recovered Shown be in the range of 98-102% the results obtained for Arterolane maleate and Piperaquine phosphate were found to be within the limits. Hence the method was found to be accurate. In the System precision study, %RSD was found to be less than 2%. For Arterolane maleate and Piperaquine phosphate within limits .which indicates that the system has good reproducibility. Retention times were found to be 3.171min and 2.338min. for Arterolane maleate and Piperaquine phosphate respectively. Arterolane maleate shows linearity in the range of 0-180ppm Limit of detection (LOD) and Limit of quantification (LOQ) were estimated from the signal-to-noise ratio. The LOD values of Arterolane maleate and Piperaquine phosphate were found to be 0.087634, 0.722994 respectively. hence a new rp-HPLC method was developed and validated.

INTRODUCTION:

Arterolane maleate Chemicaly cis-Adamantane-2-spiro-3'-8'-[[[(2'- amino-2'-methylpropyl) amino] carbonyl] methyl]-1', 2', 4- trioxaspiro [4.5] decane hydrogen maleate and its molecular formula $C_{26}H_{40}N_2O_8$ belongs to category anti malarial and soluble in water and methanol and it is acting rapidly on blood schizonticide against all blood stages of P. falciparum without effect on liver stages and acts by inhibition of PfATP6, a sarcoplasmic endoplasmic reticulum calcium ATPase encoded by P. Falciparum. Piperaquine Phosphate

chemicaly 1, 3- Bis [4- (7- chloro quinolinyl-4) - piperazinyl-1] propane tetra phosphate tetra hydrate and molecular formula $C_{29}H_{32}C_{l2}N_6$ •$4H_3PO_4$ •$4H_2O$ belongs to category Anti malarial and soluble in Water, Trifluoroacetic acid, Acetonitrile Piperaquine is a bisquinoline anti-malarial drug and shows good activity against chloroquine-resistant Plasmodium strains. Evidence suggesting the inhibition of the heme-digestion pathway in the parasite food vacuole is most convincing.

MATERIALS AND METHODS

Chemical reagents include ortho phosphoric buffer, ACN and Water of HPLC grade. Samples and standard were procured from Ranbaxy Pharma Ltd and HPLC Waters 2695 using Empower.

Preparation of mobile phase:

Buffer preparation: (0.1% OPA)

In the preparation of 0.1% ortho phosphoric buffer, 1ml of ortho phosphoric acid was diluted to 1000ml with water.

Mobile phase: Buffer and Acetonitrile taken in the ratio 70:30A

Preparation of standard stock solution:

Accurately Weighed and transferred 12mg of Arterolane and 60mg of Piperaquine working Standards into a 10ml clean dry volumetric flask, add $3/4^{th}$ volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Selection of analytical wavelength:

By appropriate dilution of standard stock solution with mobile phase, various concentrations of Arterolane maleate and Piperaquine phosphate were prepared separately. The solution was scanned using double beam UV-visible spectrophotometer 1700 in the "Spectrum mode" between the range of 400 to 200 nm and their spectra was overlaid. From the overlaid spectra of Arterolane maleate and Piperaquine phosphate, 228 nm was selected as analytical wavelength for multi component analysis using HPLC method.

Analysis of the marketed formulation.

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 900mg and transferred into a 250mL volumetric flask, 200mL of diluents added and sonicated for 25 min, further the volume made up with diluents. The solution was filtered through 0.4 µm membrane filter paper. From the filtered solution 0.4ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluents.

METHOD VALIDATION

System suitability:

Stock solution-II of (Arterolane maleate and Piperaquine phosphate) standard was injected five times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard Chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from five replicate injections.

PRECISION:

In Intraday precision the sample mixture containing 12 µg/ml of Arterolane maleate and 60 µg/ml of Piperaquine phosphate was analyzed six times at different time intervals on the same day the variation of the results within the same day was analyzed and statistically validated.

ACCURACY:

Assay was performed in triplicate for various concentrations of Arterolane maleate and Piperaquine phosphate

equivalent to 50, 100 and 150 % of the levels was injected into the HPLC system per the test procedure.

LINEARITY:

Preparation of calibration curve for Arterolane maleate and Piperaquine phosphate. Evaluation of both drugs was performed with UV detector at 228nm. Peak areas were recorded for all the peaks and peak areas were plotted against the concentrations to obtain the standard calibration curves.

SOLUTION STABILITY:

Preparated solution is kept under ideal storage conditions for 24hrs.Then the solution was injected into the chromatograph.

ROBUSTNESS

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and column oven temperature which may differ but the responses were still within the specified limits of the assay keeping flow rates 0.8ml / min and 1.2 ml / min by changing the oven temperature i.e.30^0C to 25^0C and 35^0C Standard solution was prepared and injected into the HPLC phase by changing the mobile phase i.e. 70:30 to 60:40 and 80:20.

LIMIT OF DETECTION (LOD) and LIMIT OF QUANTIFICATION (LOQ)

From the S/N ratio of the standard, LOD concentration was calculated the for S/N ratio of 3 and 10.

DEGRADATION STUDIES:

Oxidation:

To 1 ml of stock solution of Arterolane and Piperaquine, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c.

Acid and Alkali Degradation Studies:

To 1 ml of stocks solution Arterolane and Piperaquine, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 120µg/ml&600µg/ml solution and 10 µl were injected into the system.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 300µg/ml&10µg/ml&25µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º.

RESULTS AND DISCUSSION

Peaks were eluted properly, The retention time of the two peaks is less than 10 mins & Shape of two peaks is good Buffer and Acetonitrile (70: 30) detector wavelength is 228nm. From the system suitability studies it was observed that all the parameters were within limit. The variation of the results within the same day was analyzed and statistically validated. The average % recovery of both Arterolane maleate and Piperaquine phosphate was calculated and the results were summarized. A calibration curves was plotted for concentration v/s peak area. Preparated solution was kept under ideal storage conditions for 24hrs.

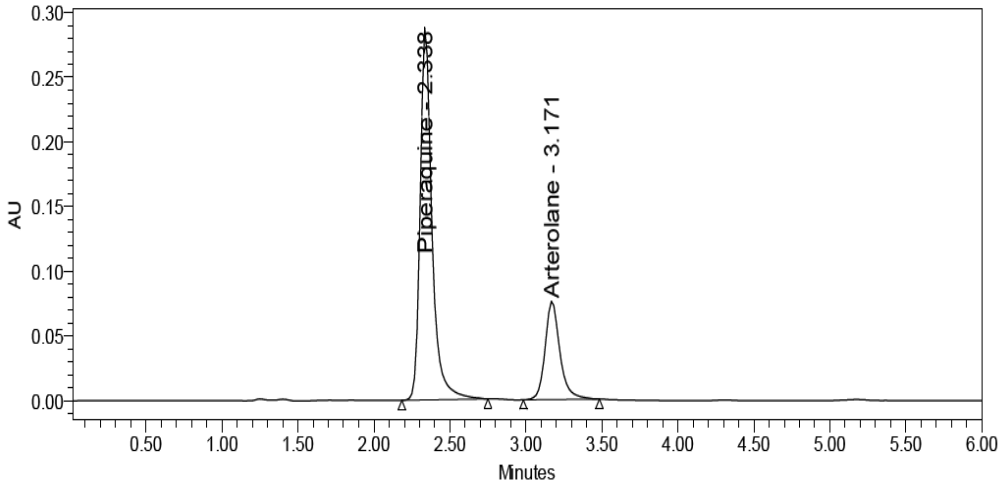

Figure 1: Chromatogram Showing Optimized method of PIP and ART.

S.NO	Retention time		Area of ART	Area of PIP	USP Plate count		USP Tailing
	ART	PIP			ART	PIP	
1	3.134	2.319	504436	1224007	5432	4586	1.15
2			507669	1234556	5558	4735	1.17
3			504931	1242950	5600	4824	1.17
4			503936	1233612	5511	4602	1.14
5			507007	1234208	5540	4662	1.16
Mean			505596	1233867			
Std. Dev.			1645.6	6715.3			
% RSD			0.3	0.5			

Table 1: Solution Stability showing Optimized method of PIP and ART.

Inj No.	Sample area ART	Sample area PIP	% Assay
1	504261	1240338	99.63
2	508217	1232739	100.41
3	510004	1230238	100.77
4	502347	1240969	99.25
5	510835	1242669	100.93
6	502328	1227452	99.25
Avg	506332	1235734	100.04
STD	3834.452	6394.745	
%RSD	0.7573	0.517486	

Table 2: Precision showing Optimized method of ART and PIP.

S.no	Spike level	Area ART	Area PIP	Tailing	% Recovery
1	50%	252212	613864	1.19	99.66
	50%	255054	620523	1.16	100.79
	50%	253523	612719	1.21	100.18
2	100%	504261	1240338	1.18	99.63
	100%	508217	1230238	1.20	100.41
	100%	505531	1226489	1.23	99.88
3	150%	760027	1860446	1.25	100.11
	150%	758190	1851686	1.25	99.87
	150%	757977	1858983	1.25	99.84

Table 3: Accuracy showing Optimized method of ART and PIP.

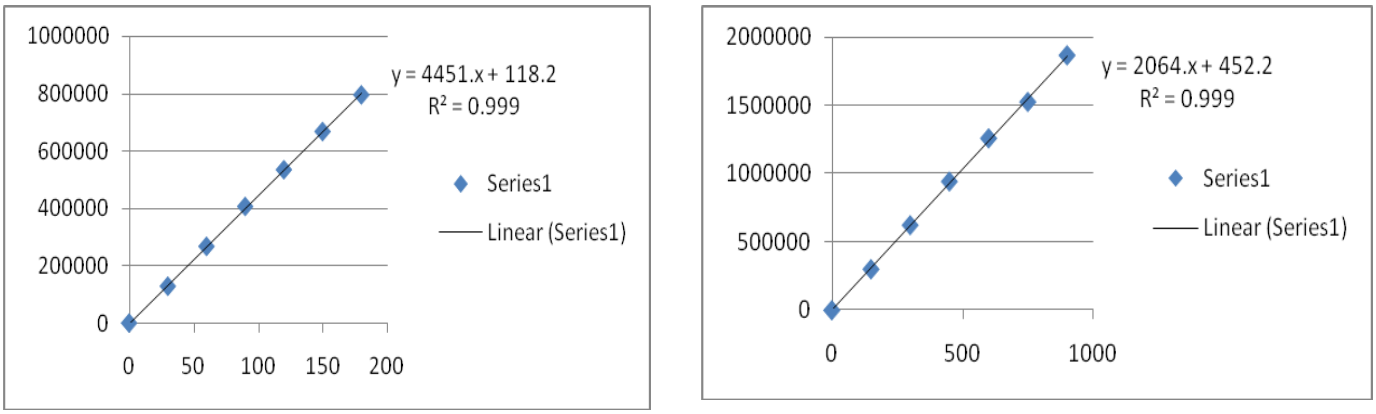

Figure 2: Calibration Curve of ART and PIP.

Method Parameter	Retention Time (min)		Tailing factor	
Flow rate (ml/min)	Arterolane maleate	Piperaquine Phosphate	Arterolane maleate	Piperaquine Phosphate
0.6	3.138	2.331	1.24	1.32
0.8	3.147	2.335	1.23	1.34

Table 4: Robustness showing Optimized method of ART and PIP.

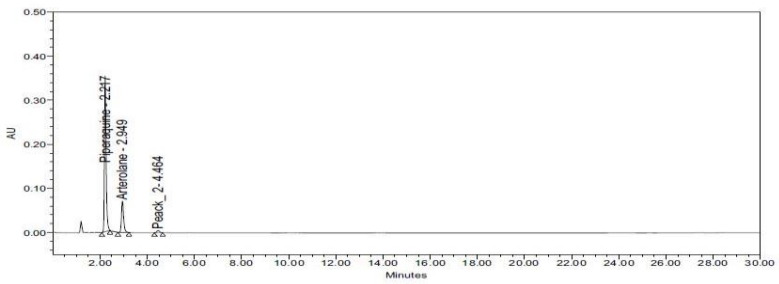

Figure 2: Chromatogram Showing Degradation studies of peroxide Sample

Then the solution was injected into the chromatograph. The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and column oven temperature which may differ but the responses were still within the specified limits of the assay. The LOD and LOQ were calculated based on S/N ratio. Forced, Dry Heat, Photo Stability and Neutral Degradation studies were also conducted for acid and alkali and results are summarised within the limit.

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