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Development and Validation of A Stability Indicating Liquid Chromatographic Method for Simultaneous Estimation of Arterolane Maleate and Piperaquine Phosphate in Combined Dosage Form

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ABSTRACT

A novel stability indicating isocratic, reversed phase-liquid-chromatographic method has been developed for the simultaneous quantitative determination of Arterolane maleate And Piperaquine phosphate in combined-dosage form. A thermo hypersil BDS C18 (250*4.6*5µ) column with mobile phase containing water pH 2.8 adjusted with ortho phosphoric acid: methanol in the ratio of (60: 40, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 241 nm. The retention times of Arterolane Maleate and Piperaquine Phosphate were 1.864min and 3.047min, respectively. Correlation co-efficient for Arterolane Maleate and Piperaquine Phosphate was found to be 0.99 and 0.99, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Arterolane Maleate and Piperaquine Phosphate in formulations was found to be in the range of 97-103% and 97-103% respectively confirms the non-interferences of the excipients in the formulation. Arterolane Maleate and Piperaquine Phosphate were exposed to stress conditions like acidic hydrolysis, basic hydrolysis, oxidative, photolytic, humidity and thermal conditions. Due to its simplicity, rapidness and high precision, the method was successfully applied to the estimation of Arterolane Maleate and Piperaquine Phosphate in combined dosage form.

Key words: Arterolane Maleate, Piperaquine Phosphate, Method validation, Forced degradation.

INTRODUCTION

Malaria is a major public health problem infecting 300-500 million people worldwide and causing more than one million deaths annually globally. An endemic protozoal disease in India with an estimated 70-100 million cases each year, Plasmodium falciparum is responsible for half of these cases. Fixed dose combination of arterolane maleate (150 mg) and piperaquine phosphate (750 mg) has been recently approved by FDA as a once-a day therapy for three days for the treatment of acute, uncomplicated Plasmodium falciparum malaria[3].

Arterolane maleate (AM), chemically [(N-(2-amino-2-methylpropyl)-2-cis-dispiro(adamantane-2,3'-[1,2,4]trioxolane-5',1"-cyclohexan)-4"-yl] acetamide is a synthetic trioxolane, easy to synthesize, affordable and rapidly acting oral antimalarial. It is a rapidly acting blood schizonticide against all blood stages of P. falciparum without effect on liver stages. Arterolane is an active moiety which gets accumulated either in cytosol or food vacuole of the parasite. It acts by inhibition of PfATP6, a sarcoplasmic endoplasmic reticulum calcium ATPase encoded by P. Falciparum. In the food vacuole of parasite reductive cleavage of peroxide bond of arterolane by ferrous iron (fenton reaction) occurs. This irreversible redox reaction produces free radicals that alkylate the membrane associated parasite proteins. The reactive species inhibits an ATP-dependent Ca2+ pump[1] located on the endoplasmic reticulum, PfATP6. The pump, called PfATP6, is a homologue of a mammalian sarcoplasmic /endoplasmic reticulum Ca2+ ATPase (SERCA). The reactive C radicals are thought to subsequently react more or less indiscriminately with different protein targets as well as with ferriprotoporphyrin IX itself, thus preventing heme detoxification and inhibiting a multitude of enzymes. Piperaquine phosphate (PIP), chemically 1,3-bis-[4-(7-chloroquinolyl-4)-piperazinyl-1]-propane phosphate, is 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. Piperaquine's bulky bisquinoline structure may be important for activity against chloroquine resistant strains and may act by inhibition of the transporters that efflux chloroquine from the parasite food vacuole. Arterolane kills the malaria parasite in the blood, providing fast relief from symptoms of malaria like fever and chills. Piperaquine, on the other hand, has a longer-lasting effect than arterolane and kills residual parasites, preventing the recurrence of malaria.

A literature survey revealed few liquid chromatography (LC) assay methods that have been reported for the determination of Arterolane in bulk dug and pharmaceutical dosage forms, but there are no reported methods for simultaneous estimation of Arterolane maleate and Piperaquin phosphate in combined pharmaceutical dosage forms[3-11].

The present International Conference on Harmonization (ICH) drug stability guidelines suggest that stress studies should be conducted on the drug product to establish its inherent stability characteristics, and the analytical method should able to separate all degradation impurities formed under stress studies to prove its stability-indicating power. In order to monitor possible changes to a product over time, the applied analytical chromatographic method must be stability-indicating. The best case for testing the suitability of a method is using real-time stability samples containing all relevant degradation products that might occur. But due to product development timelines, process characteristics, excipients, and other environmental factors, a forced degradation study (stress test) can serve as an alternative.

The aim of the present work is to focus on the development of an efficient stability indicating liquid chromatographic method for simultaneous estimation of Arterolane Maleate and Piperaquine Phosphate in combined pharmaceutical dosage form such as Tablets in presence of its excipients and degradation products in a short chromatographic run.

EXPERIMENTAL

Materials and methods

Instrumentation

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, and Waters Empower2 software and thermo hypersil BDS column (250mmx4.6mm, particle size 5µm).

Chemicals and Reagents

Arterolane Maleate and Piperaquine Phosphate were taken as a gift sample from Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions

The mobile phase consisting of water (pH 2.8 adjusted with orthophosphoric acid) and methanol (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were

pumped from the solvent reservoir in the ratio of 60:40 v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 241nm and the run time was 5min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Preparation of Standard solution

Accurately weighed quantity, 150.0 mg of Arterolane maleate and 750mg of Piperaquine Phosphate was transferred into a 100 ml of volumetric flask and 30 ml of water was added followed by sonication for 15 minutes and make up the volume with water. 5 ml of above solution was taken into 25ml volumetric flask and diluted to the mark with water.

Preparation of Sample solution

Accurately weighed quantity, 1320mg of sample powder was transferred into 100ml of volumetric flask added 25ml of water and sonicated for 30mins and make up the volume with water and filtered through the 0.45µm filter paper Transfer above solution 5ml into 25 ml volumetric flask and make up the volume with water.

Statistics

Results are presented as the mean±SD and results were analyzed using Excel® 10, and Chromeleon®. A p value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

The analytical procedure for the estimation of Arterolane maleate and Piperaquine phosphate in marketed formulation was optimized with a view to develop a precise and accurate assay method. Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase containing water (pH 2.8 adjusted with orthophosphoric acid) Buffer: Methanol in the ratio of 60:40 (v/v) gave a better resolution. Using UV-visible PDA detector at 241 nm carried out the detection. Amongst the several flow rates tested, the flow rate of 1 ml/min was the best for all the drugs with respect to location and resolution of peaks. The retention time of Arterolane maleate and Piperaquine phosphate was found to be 1.864min and 3.047min respectively. The chromatograms of standard and sample solution of Arterolane maleate and Piperaquine phosphate were shown in figure 02 and 03 .The asymmetry factor of Arterolane maleate and Piperaquine phosphate was 0.984 and 0.989 found to be respectively, which indicates symmetrical nature of the peak. The percentage label claim of individual drugs found in formulations were calculated and presented in table

Table 1: Table for Assay

Sample	Label claim (mg/tablet)	Amount present (mg/tablet)	%w/w
Arterolane	150	149.11	99.1
Piperaquine	750	748.05	99.7

Table 2: System Suitability Parameters

Parameters	Arterolane	Piperaquine
Correlation Coefficient (r^2)	0.99	0.99
Regression Equation	y = 16616x	y = 19288x
LOD	2.980	2.909
LOQ	9.934	9.696
Theoretical plates	3483	3619
Tailing	1.201	1.103

01.The results of analysis shows that the amounts of drugs estimated were in good agreement with the label claim of the formulations.

Method validation

System Suitability[12] Studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions

Table 3: Precision Studies

S. No	Sample wt.	Area (AM)	Area(PIP)	%Assay (AM)	%Assay (PIP)
1	1320	6072665	10312795	99	99
2	1320	6071025	10322537	99	99
3	1320	6079077	10334225	99	99
4	1320	6073043	10342014	99	99
5	1320	6071349	10364416	99	99
6	1320	6073332	10386481	99	100
Average	99	99			
STD	0.05	0.26			
% STD	0.05	0.26			

Table 4: Accuracy for Arterolane maleate

Spiked Level (%)	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	Mean
50	660.0	3036395	148.5	148.48	100	100
50	660.0	3034859	148.5	148.40	100	
50	660.0	3037123	148.5	148.51	100	
100	1320.0	6076356	297.0	297.13	100	100
100	1320.0	6074904	297.0	297.06	100	
100	1320.00	6072926	297.0	296.96	100	
150	1980.0	9118042	445.5	445.86	100	100
150	1980.0	9118924	445.5	445.91	100	
150	1980.0	9119797	445.5	445.95	100	

Table 5: Accuracy for piperaquine phosphate

Spiked Level (%)	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	Mean
50	660.00	5191132	750.000	748.05	100	100
50	660.00	5195262	750.000	747.76	100	
50	660.00	5190982	750.000	748.19	100	
100	1320.00	10366711	1500.000	1490.81	99	99
100	1320.00	10362033	1500.000	1490.14	99	
100	1320.00	10327865	1500.000	1485.22	99	
150	1980.00	15550253	2250.000	2236.24	99	99
150	1980.00	15591664	2250.000	2242.19	100	
150	1980.00	15509884	2250.000	2230.43	99	

(Table 02). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method**.**

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. The specificity was established by preparing an Arterolane Maleate and Piperaquine Phosphate standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure.

Accuracy and precision

The accuracy of the method was determined by recovery experiments. From the data obtained, added recoveries of standard drugs were found to be accurate (Table 04 & 05). Repeatability was checked

Table 7: Robustness for Arterolane maleate

Sample Name	RT	Area	USP Tailing	USPPlate count	S/N
Temp-1	1.958	6009077	1.289	3830	401.39
Temp-2	1.762	6053043	1.256	3812	427.34
Flow-1	1.960	6022665	1.256	3935	440.87
Flow-2	1.761	5871025	1.239	3912	402.06

Table 8: Robustness for piperaquine phosphate

Sample Name	RT	Area	USP Tailing	USPPlate count	S/N
Temp-1	3.138	10334225	1.185	3963	431.83
Temp-2	2.939	10342014	1.185	3976	464.09
Flow-1	3.141	10312795	1.173	3123	477.01
Flow-2	2.944	10122537	1.139	3008	444.11

Table 9: Degradation studies for Arterolane maleate

Stress condition	Sample weight	Area	%Assay	%Deg
Acid	1320	8939461	80	14
Base	1320	8259004	79	21
Peroxide	1320	8739307	84	16
Heat	1320	9509080	96	9
Light	1320	9600182	95	8

Table 10: Degradation studies for Piperaquine phosphate

Stress condition	Sample weight	Area	%Assay	%Deg
Acid	1320	4922982	80	19
Base	1320	4855195	79	20
Peroxide	1320	5131608	84	15
Heat	1320	5926300	96	3
Light	1320	5843103	95	4

by injecting six individual sample preparations. Percent RSD of the area for each drug was calculated (Table-03).

Linearity and range

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 16616x (R^2=0.99) for Arterolane maleate and y = 19288x (R^2=0.99) for piperaquine phosphate. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration

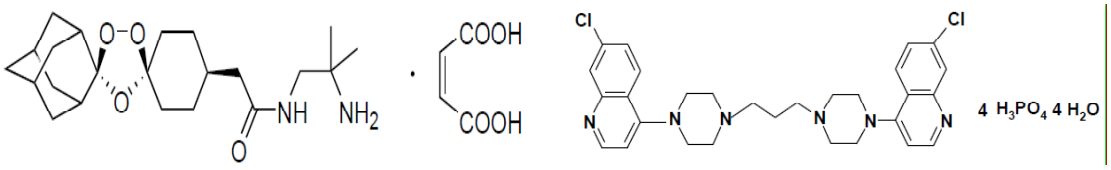

Fig. 1: Structure of Arterolane Maleate and Piperaquine Phosphate

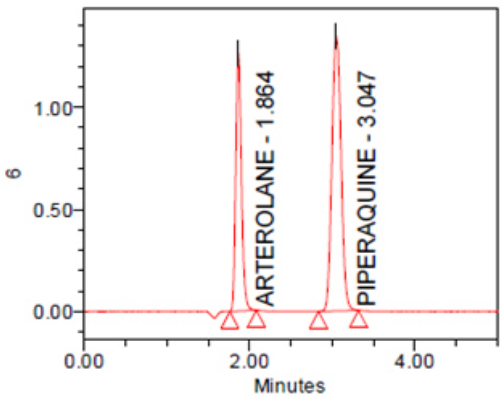

Fig. 2: Standard chromatogram for Arterolane and piperaquine

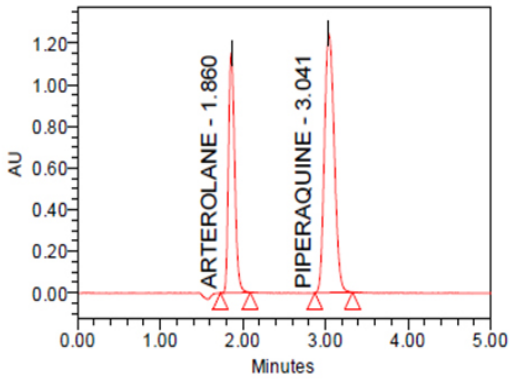

Fig. 3: Formulation chromatogram for Arterolane and piperaquine

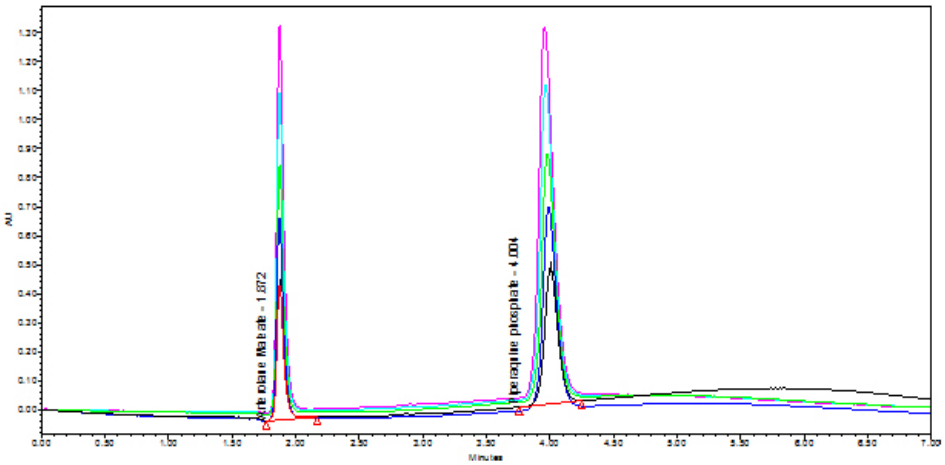

Fig. 4: Overlay chromatograms of Linearity for Arterolane and Piperaquine

range indicated above. The overlay chromatograms of Linearity for Arterolane maleate and piperaquine phosphate shows in Fig 4 and the results for calibration curves are given in Fig 5.

Limit of detection & limit of quantification (LOD & LOQ)

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Arterolane maleate and piperaquine phosphate. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b).

(a) LOQ = 10 ó / S

(b) LOD = 3.3 ó / S

where

σ = residual standard deviation of response

S = slope of the calibration curve

LOD and LOQ for Arterolane maleate and Piperaquine phosphate are found to be 2.980, 9.934 and 2.909, 9.696 respectively (Table 06).

Robustness

Robustness of the method was determined by making slight changes in the chromatographic Conditions. It was observed that there were no marked changes in the chromatograms, which Demonstrated that the RP HPLC method developed is rugged and robust (Table 07 & 08).

Forced degradation studies:

The stability studies were determined by applying the physical stress (acid, base, peroxide, water and light) to the product. It was observed that there were marked degradation in the chromatograms, and the data given in table-09 & 10).

Based on the results of the stress studies, the degradation behavior of Arterolane Maleate and Piperaquine Phosphate is as follows.

Acid degradation

Arterolane maleate and Piperaquine Phosphate were stable to degradation in 5 N HCl at 70°C for 10 min moderately. The impurities formed during this study are well separated from main drug peaks and mass balance is found to be in acceptable limit. Peak purity of drugs also matches (Fig. 06).

Base degradation

Arterolane Maleate and Piperaquine Phosphate were found to be slightly unstable in 5 N NaOH at 70°C for 5 min. The major degradation peaks are well separated from drug peaks and well resolved. Mass balance is found to be in acceptable limit. Peak purity of drugs also matches (Fig. 6).

Oxidation degradation

Arterolane Maleate and Piperaquine Phosphate were found to be slightly unstable under conditions of 3% hydrogen peroxide at 70°C for 10 min. The major impurities in the study were resolved with drug peaks. Mass balance is found to be in acceptable limit. Peak purity of drugs also matches (Fig. 06).

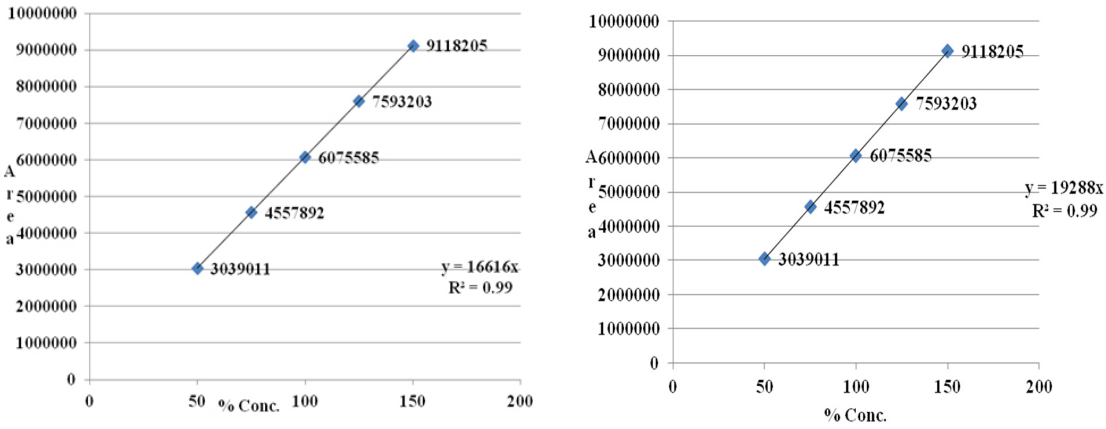

Fig. 5: Linearity Curve for Arterolane and Piperaquine

Thermal degradation

Arterolane Maleate and Piperaquine Phosphate were found to be stable to thermal exposure. Partial degradation was take place. Impurities formed well resolved from main drug peaks. Mass balance is found to be in acceptable limit. Peak purity of drugs also matches (Fig. 6).

Photolytic degradation

Upon subjecting Arterolane Maleate and Piperaquine Phosphate samples to both UV and visible light, only partial degradation of was observed (Fig. 6).

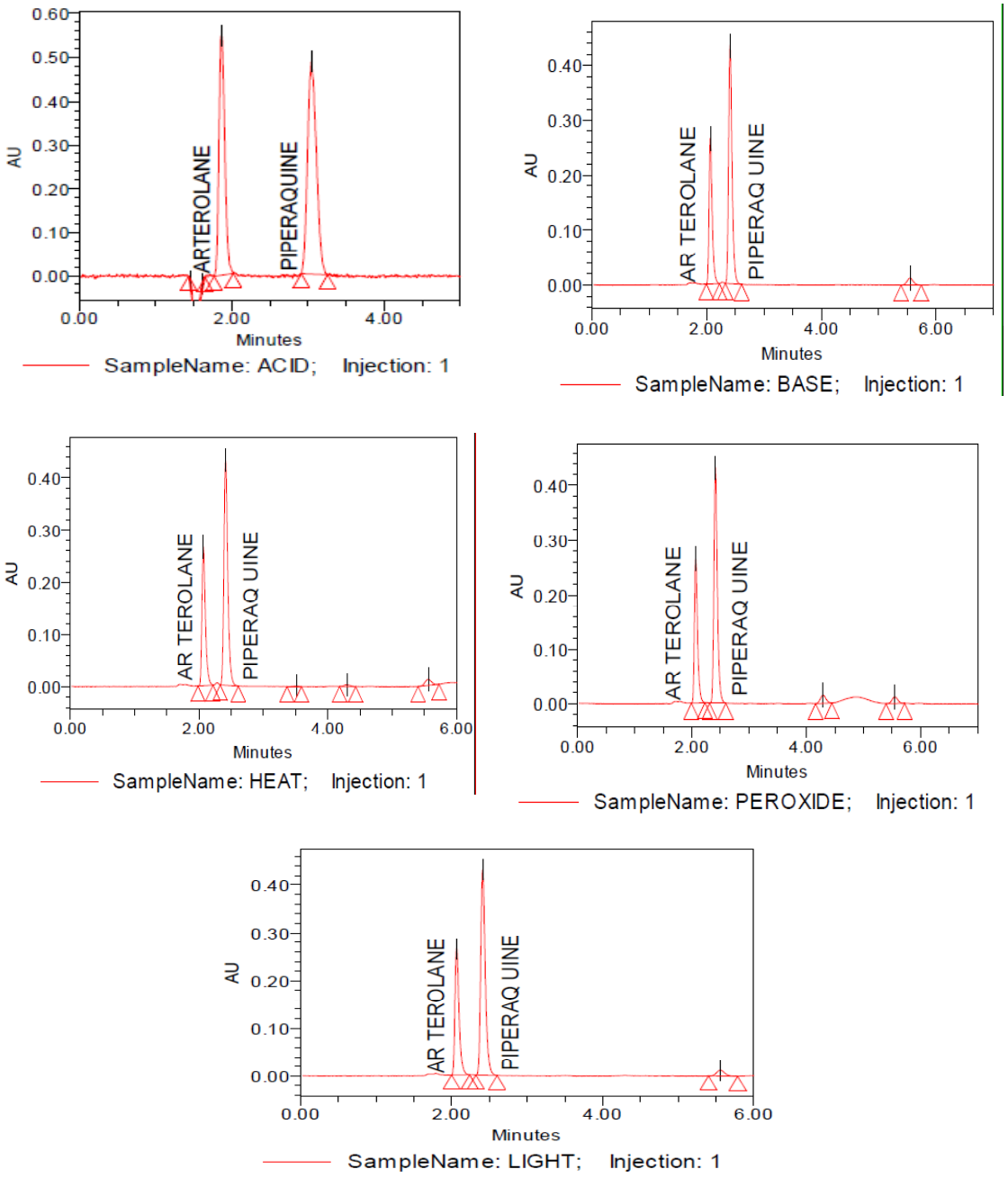

Fig. 6: Typical chromatograms of Forced degradation studies

Testing of a placebo containing preservative leads to formation of number of different impurities with respect to an unstressed placebo. The amount of preservative decreased mainly by influence of oxidation, light and acid. Mass balance of preservative shows almost 100%. The active ingredients remain almost stable within tested period and mass balance matches (Table 9 & 10).

System suitability results were given by table 01 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Arterolane maleate and piperaquine phosphate standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method precision passed for both Arterolane maleate and piperaquine phosphate studies. The method accuracy was evaluated by recovery studies. Arteorlane maleate and piperaquine phosphate recovery was found to be 100% as per ICH 97%-103% and also percentage RSD was very low so method is accurate shown in table 4&5. Linearity calibration curve was given below fig: 5 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation coefficient. Linear correlation was found to be Y= 16616 for Arterolane maleate and y = 19288 for piperaquine phosphate the intraday and inter day variations was calculated in terms of %RSD and results was found to be intraday and inter day respectively. Method robustness results were given by table 7 & 8. Stability studies are given in Table 9 & 10.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Arterolane maleate and piperaquine phosphate in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Arterlane maleate and piperaquine phosphate in pure and its pharmaceutical dosage forms.

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