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A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PIPIERAQUINE PHOSPHATE AND ARTEROLANE MALEATE BY RP-HPLC METHOD

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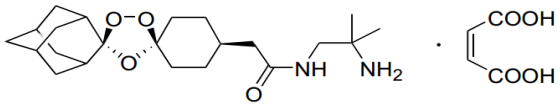
ABSTRACT

A simple, economical, precise, accurate and rapid HPLC method has been developed and validated for assay determination of Piperaquine Phosphate and Arterolane maleate simultaneously in their tablet dosage forms. The chromatographic conditions was performed using Waters Hypersil ODS (150*4.6mm*5µ) Column on a mixture of Methanol:0.01M Potassium dihydrogen phosphate (pH 2.6) in the ratio of (60:40%v/v). The detection was carried out at 240nm with the flow rate of 1.0 ml/min. The retention times for PQP, AM were 3.39 and 5.53 min respectively. This method shows to be Linear (R^2>0.99), precise (RSD<2%), Accurate Recovery of (98-102%) of PQP and AM. The proposed method was successfully employed for the drug contents in marketed formulations, according to ICH guidelines and found to be suitable for the simultaneous estimation of the drugs.

Key words: Piperaquine Phosphate, Arterolane maleate, RP-HPLC, Combined dosage form.

1. INTRODUCTION

Piperaquine Phosphate is chemically known as 7-chloro-4-[4-[3-[4-(7- Chloroquinolin-4-yl) piperazin-1-yl]prppyl] piperazin-1-yl]quinolne; phosphoric acid. Arterolane maleate is chemically known as [(N-(2-amino-2-methyl propyl)-2-cis-(adamantine-2,3'-[1,2,4] trioxolane-5',1"-cyclohexan)-4"-yl] acetamide: maleate (Tripathi KD.,2008). PQP and AM acts as Anti-Malarial drugs. PQP interferes with the degradation of haemoglobin by parasitic lysosomes, helps in damaging of plasmodial membranes. AM causes lipid peroxidation, damages endoplasmic reticulum, inhibits protein synthesis and ultimately results in lysis of the parasite[3]. Highly sensitive, selective, HPLC method will be very useful for the estimation of PQP and AM in combined dosage formulations. Literature survey reveals need for simultaneous estimation of drugs. Few methods were reported by HPLC, LC-MS, Capillary zone electroporosis. The purpose of this study was to develop sensitive, simple, precise, accurate method for simultaneous estimation of PQP and AM in bulk and combined dosage form (Debrus B., 2011).



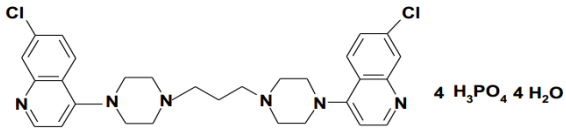


Figure.1.Chemical Structure of Piperaquine phosphate **Figure.2.Chemical Structure of Arterolane maleate**

MATERIALS AND METHODS

Apparatus: Separation and estimation was carried out using HPLC (waters-2469 with PDA detector), column used in experiment was C_{18} Waters Hypersil ODS, 5µ(150*4.6mm) analytical balance used was LAB INDIA, Digital pH meter LAB INDIA. The mobile phase was prepared by mixing 0.01M Potassium dihydrogen phosphate buffer: Methanol(pH-2.6) in the ratio of (60:40) was filtered and degassed. Injection volume is 10µL and the detection was at 240nm.

Reagents and solutions: Pure sample of Piperaquine phosphate and Arterolane maleate and other reagents such as Methanol, milliQ water of HPLC grade and Potassium dihydrogen phosphate.

Preparation of mobile phase: The mobile phase was prepared by mixing 0.01M Potassium dihydrogen phosphate: Methanol (pH-2.6) in the ratio of (60:40%v/v) and was filtered and degassed.

Preparation of standard drug solution: About 15.0 mg of AM, 75.0 mg of PQP standard were weighed accurately into 100ml of volumetric flask. Add 70ml of Diluent was added and sonicated for 5 minutes. After sonication, the volume was made up to the mark with diluent to obtain final concentration of 150 µg/ml, 750 µg/ml AM and PQP respectively. (Wahajuddin., 2013) (Tarning J., 2006).

Marketed Formulation: 20 tablets were accurately weighed and average weight was determined. Powdered tablet equivalent to 150 mg of AM and 750mg of PQP was transferred into a 100 ml volumetric flask. 70 ml of water was added and sonicated for 10 minutes. The volume was made upto mark with diluents after cooling and mixed well, filtered through 0.45 µ nylon filter. Finally get concentration of AM as about 1500µg/ml, PQP 7500µg/ml. The solution was

filtered through 0.45µ nylon filter. Further 5ml from this solution was diluted to 50 ml with diluent and mixed well to get concentration of 150µg/ml of AM, 750µg/ml of PQP. (Hodel E., 2009) (Kirchhofer C., 2010)

Table.1.Details of marketed Formulation

Brand Name	Content	Mfg. Company
Synriam	Piperaquine Phosphate& Arterolane maleate(750 mg & 150mg Respectively)	Ranbaxy

Selection of mobile phase for method Optimization and experimental condition: Several trial had been taken for the proper optimization of HPLC method by changing different mobile phase with different ratio. And finally the mobile phase for optimised condition 0.01 M potassium dihydrogen phosphate: Methanol (pH-2.6) in the ratio of (60:40) was selected and chromatogram was shown Fig no.3.

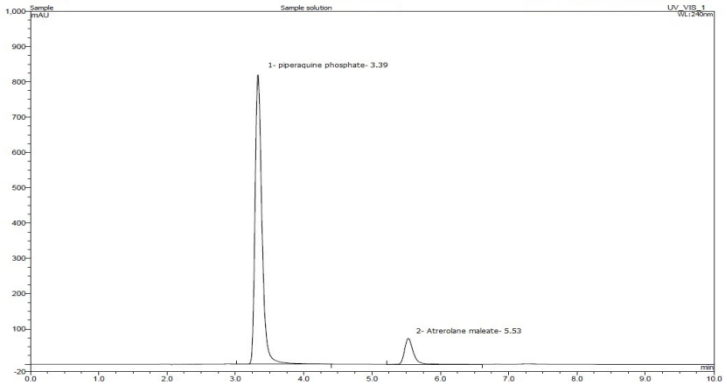


Figure.3.Chromatogram of PQP and AM for optimised method

Table: 2 Chromatographic conditions for the optimized method

Parameters	Description
Column	Waters Hypersil ODS C18 (150*4.6mm,5.0µ)
Mobile phase	0.01 M Potassium dihydrogen Phosphate : Methanol (60:40)
Injection volume	10 µl
Flow rate	1.0 ml/min
Detection wavelength	240nm
Column Temparature	40°C
Sample Temparature	25°c
Run Time	10 min

Assay: Assay of marketed formulation containing PQP (750mg) and AM(150 mg) was performed by preparing the sample solution as describer earlier in the preparation of the sample. The assay of the commercial sample was calculated by comparing the area of standard and sample peaks. The assay of marketed formulation synriam was found to be within the limit; the chromatogram is shown in Fig 4.

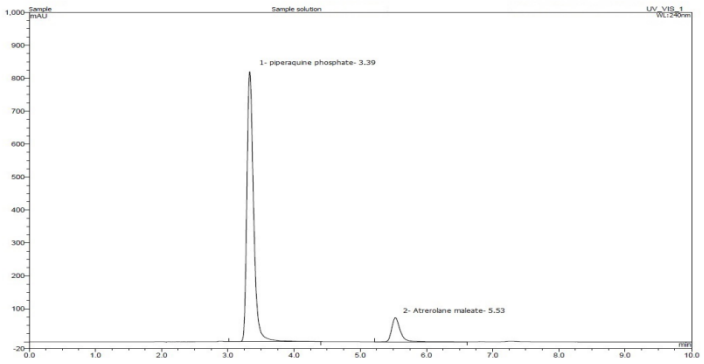


Figure.4.Chromatogram for the Assay of marketed Formulation

This optimized method was validated terms of Linearity, accuracy, Precision, Specificity, as per ICH guidelines.

Linearity: The linearity of calibration curves (peak area V_s concentration) in pure solution was checked over the concentration ranges of about 50-150% (Assay concentration µg/ml) for PQP and AM. The total eluting time was less than 10.0 min. The calibration curve were linear in the studied range and equations of the regression analysis were obtained y=-0.1152x, R^2=0.999 for PQP and y=-0.7204.x, R^2=0.999 for AM.

Accuracy: Accuracy of the method was determined by recovery experiments at spiked levels of 50%, 100%, 150%. The recovery studies were carried out three times, the percentage recovery and percentage relative standard deviations were calculated.

Precision: The precision of the analytical method was studied by analysis of multiple sampling of same homogeneous sample.

Intra-day Precision: Intra-day precision was determined by analyzing the combined standard solutions of AM and PQP (150,750 µg/ml) at three different time intervals on same day.

Inter-day Precision: Inter-day precision was determined by analyzing the combined standard solutions of AM and PQP (150,750 µg/ml) on three consecutive days.

Specificity: A solution containing a mixture of tablet was prepared using sample preparation procedure and injected in to the system , to evaluate possible interfering peaks.

Forced degradation: Forced degradation studies were carried out on the sample preparations of AM, PQP tablets 150:750 mg and the degradation was evaluated by calculating the % degradation of AM and PQP in comparison with unstressed sample preparation. It was tried to achieve degradation of these drug between 10 % and 30% by following stress conditions to prove the method as a stability indicating method.

The following are the stress conditions which were followed for forced degradation studies;

Acid Degradation: Weigh accurately about 1368mg of Sample in 100ml volumetric flask and 20ml of diluents and add 5ml of 5N HCl boil for 1hr (80^0c) and neutralize with 5N NaOH and make up to the mark. 5ml of this solution is diluted in to 50ml and filtered through the 0.45 nylon filtered and analyse the recorded chromatogram.

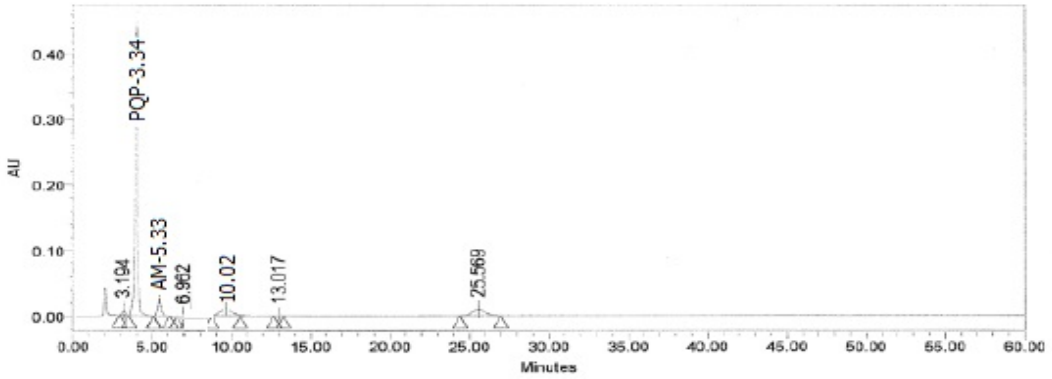


Fig No.5:Chromatogram of Combined Tablet Solution of PQP, AM in Acid Stress Degradation

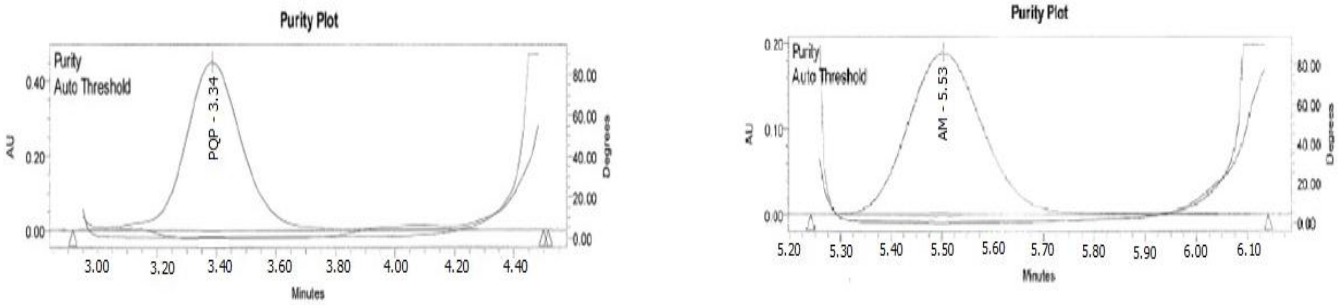


Figure.6.Chromatogram of Purity Plot of PQP and AM in Acid Stress Degradation

Alkali Degradation: Weigh accurately about 1368mg of Sample in 100ml volumetric flask and 20ml of diluents and add 5ml of 5N NaOH boil for 1hr (80^0c) and neutralize with 5N HCl and make up to the mark. 5ml of this solution is diluted in to 50ml and filtered through the 0.45 nylon filtered and analyse the recorded chromatogram.

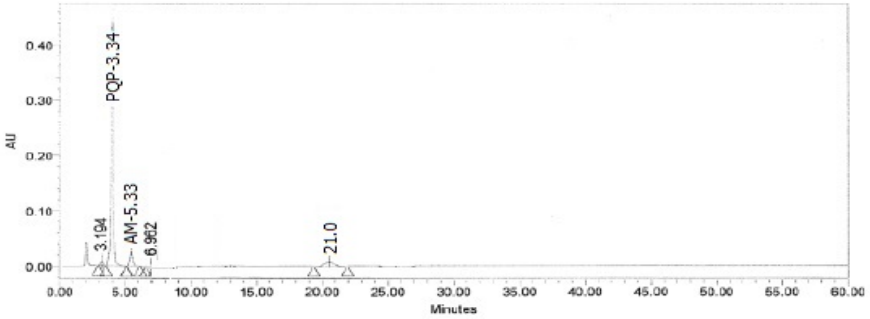


Figure.7.Chromatogram of Combined Tablet Solution of PQP and AM in Alkali Stress Degradation

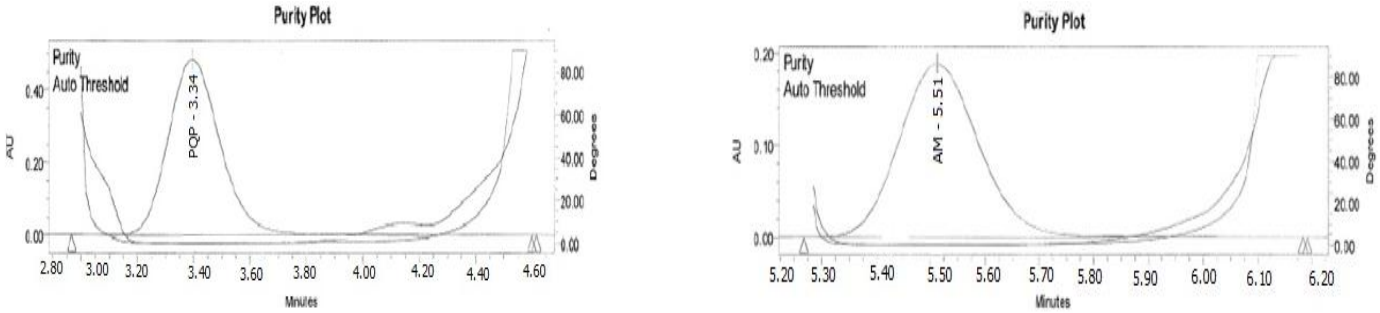


Figure.8.Chromatogram of Purity Plot of PQP & AM in Alkali Stress Degradation

Peroxide Degradation: Weigh accurately about 1368mg of Sample in 100ml volumetric flask and 20ml of diluents and add 5ml of 6% H_2O_2 boil for 1hr (800c) and make up to the mark. 5ml of this solution is diluted in to 50ml and filtered through the 0.45 nylon filtered and analyse the recorded chromatogram.

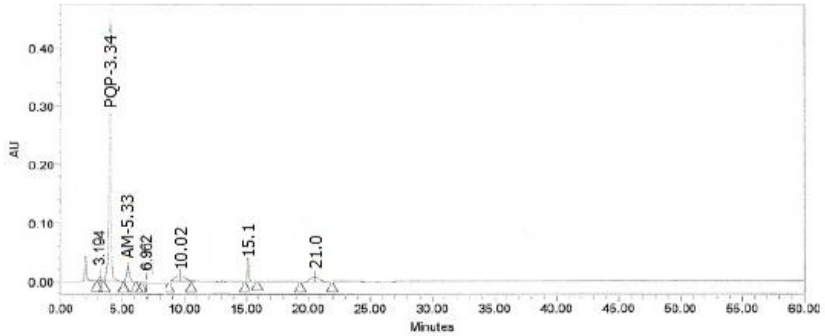


Figure.9.Chromatogram of Combined Tablet Solution of PQP and AM in Peroxide Stress Degradation

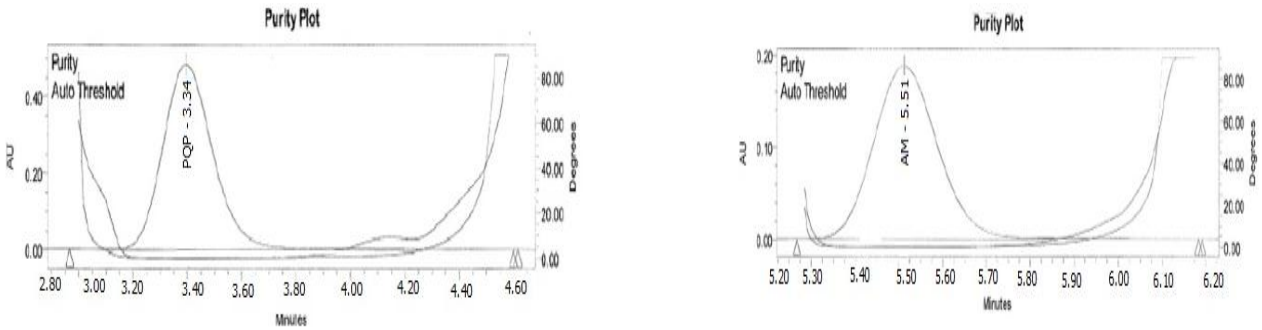


Figure.10.Chromatogram of Purity Plot of PQP in Peroxide Stress Degradation

RESULTS AND DISCUSSION

Validation:

Table.3.Method Precision Results for Piperaquine Phosphate

Test No.	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Avg.Wt. (mg)	**1368.23**					
Wt. taken (mg)	1368.01	1359.98	1367.98	1368.26	1368.00	1368.35
Area (Inj.1)	8563656	8568156	8571651	8566376	8563546	8565654
Area (Inj.2)	8563685	8565658	8553658	8566686	8559696	8561686
Avg. Area	8563670	8563457	8563465	8566321	8561236	8562315
% RSD	0.1	0.2	0.1	0.2	1.1	0.1
Assay(mg/tab)	750.52	750.70	751.06	750.09	750.92	750.62
Assay (%)	100.1	101.2	101.8	100.2	101.9	100.9
Average Assay	In mg= 750.65mg		In %=100.92%			
STDV	For mg assay=0.6141		For % assay=0.4262			
%RSD	For mg assay=0.4		For % assay=0.4			

Table.4. Method Precision Results for Arterolane maleate

Test No.	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Avg.Wt. (mg)	**1369.05**					
Wt.taken(mg)	1368.75	1369.25	1368.95	1368.05	1367.95	1368.55
Area (Inj.1)	1132502	1132512	1132562	1140118	1132758	1132856
Area (Inj.2)	1132635	1140212	1136574	1139925	1135555	1132641
Avg. Area	1132569	1136362	1134568	1140022	1134157	1132749
% RSD	0.2	0.1	0.0	0.2	1.1	0.1
Assay(mg/tab)	151.06	151.15	151.09	152.03	151.09	151.05
Assay (%)	100.9	101.2	101.0	101.9	101.0	100.8
Average Assay	In mg= 151.28mg		In %=101.4%			
STDV	For mg assay=1.3058		For % assay=0.4243			
%RSD	For mg assay=0.4		For % assay=0.4			

Table.5.Result of Intraday and Interday precision of PQP(750µg/ml)

Conc. µg/ml	Time (hr.)	Mean Peak Area n=6	%RSD	Time (days)	Mean Peak Area n=6	% RSD
750(PQP)	0	8569654	0.08%	1	8569654	0.12%
750 (PQP)	2	8569364	0.08%	2	8559364	0.07%
750 (PQP)	4	8568965	0.11%	3	8558965	0.19%

Table.6.Result of Intraday and Interday precision of AM (150µg/ml)

Conc. µg/ml	Time (hr.)	Mean Peak Area n=6	%RSD	Time (days)	Mean Peak Area n=6	% RSD
150 (AM)	0	1132502	0.17%	1	1132512	0.16%
150 (AM)	2	1132546	0.11%	2	1102296	0.11%
150 (AM)	4	1129956	0.17%	3	1128250	0.17%

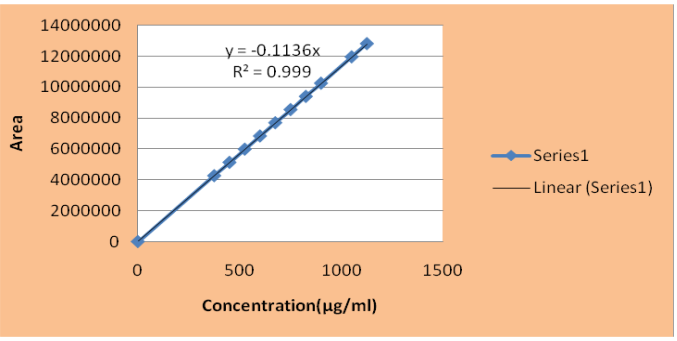
System suitability tests were carried out on freshly prepared standard solution and all the parameters are within limit. Results were shown in table No:7

Table.7. System suitability data

Parameters	**PQP** (± %RSD)	**AM** (± %RSD)
Retention Time	3.39±0.13	5.53±0.14
Theoretical plate	4035±0.12	3025±0.34
Tailing Factor	1.05±0.12	1.16±0.23

± %RSD = Percentage Relative Standard Deviation.

The method was linear in the concentration range of 375-1125µg/ml for PQP and 75-225µg/ml for AM, with a correlation coefficient of 0.999 for PQP and 0.999 for AM.



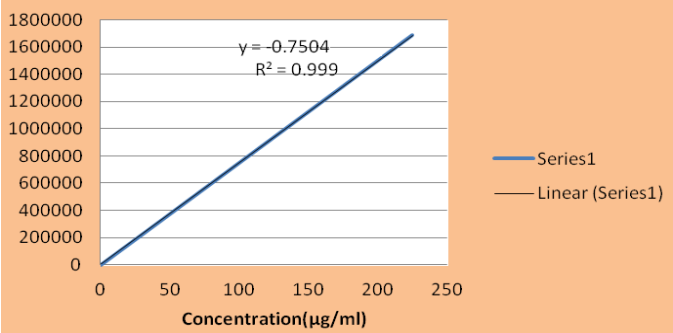


Figure.11.Linearity plot of PQP **Figure.12.Linearity plot of AM**

The study of accuracy of the developed method has been done. The recovery was found in the range of 101.2%for PQP and 100.7%for AM shown in Table.8, indicating the accuracy of method and the % RSD of PQP and AM is 0.50 and 0.50 respectively.

Table.8.Accuracy Data Of The Analysis Of Pqp And Am

Concentration Of Spiked level %	Amount Std added µg/ml		Total amount found µg/ml		% Recovery µg/ml		Mean	
	PQP	AM	PQP	AM	PQP	AM	PQP	AM
50	375.2	75.1	374.9	74.8	101.4	101.3	101.2	101.7
100	750.3	150.2	749.8	149.9	101.4	101.3		
150	1125.2	225.1	1124.9	224.7	101.3	100.7		

Table No: 9 Result of Peak Purity of Drug in Acid Stressed Degradation

Name of Drug	Peak Purity Result		
	Purity Angle	Purity Threshold	Conclusion
Piperaquine phosphate	0.048	0.214	Passed
Arterolane maleate	0.217	0.232	Passed

Table.10.Result of Peak Purity of Drug in Alkali Stressed Degradation

Name of Drug	Peak Purity Result		
	Purity Angle	Purity Threshold	Conclusion
Piperaquine phosphate	0.049	0.230	Passed
Arterolane maleate	0.063	0.234	Passed

Table.11.Result of Peak Purity of Drug in Peroxide Stressed Degradation

Name of Drug	Peak Purity Result		
	Purity Angle	Purity Threshold	Conclusion
Piperaquine phosphate	0.057	0.209	Passed
Arterolane maleate	0.120	0.233	Passed

Forced degredation were carried out on prepared sample solution and all the parameters are within limit i.e10-30% of degredation and purity angle of the peak purity should be less than the purity threshold. Results were shown in table No:12

Table.12.Result of Stress Study for PQP

Standard Area				
8563656	8568156	8571651	8566376	8563546
Average Area	8566677			
%RSD	0.3%			
Test No.	**Unstressed**	**Acid Stress**	**Alkali Stress**	**Peroxide Stress**
Avg. Wt (mg)	1368.90			
Wt. taken (mg)	1369.12	1367.90	1368.5	1368.1
Area (Inj.1)	8533457	7748379	7671577	6212356
Area (Inj.2)	8536557	7745630	7670521	6218563
Avg. Area	8561457	7746869	7671528	6215369
% RSD	0.1	0.6	0.1	0.7
Assay(mg/tab)	751.20	682.08	675.32	546.87
Assay (%)	100.8	90.8	89.9	72.8
% Degradation	NA	9.9	10.8	27.8

Table.13.Result of Stress Study for AM

Standard Area				
1125632	1126325	1125325	1125625	1125865
Avg area	1125754			
%RSD	0.2%			
Test No.	**Unstressed**	**Acid Stress**	**Alkali Stress**	**Peroxide Stress**
Avg. Wt. (mg)	1368.92			
Wt.taken(mg)	1368.10	1368.90	1368.5	1368.4
Area (Inj.1)	1132632	1055269	1062062	1024698
Area (Inj.2)	1131642	1056325	1063256	1025636
Avg. Area	1132263	1055865	1062965	1024953
% RSD	0.1	0.5	0.0	0.7
Assay (mg/tab)	150.00	138.69	139.58	134.67
Assay (%)	100.8	93.2	93.8	90.5
Degradation(%)	NA	6.7	7.5	10.2

4. CONCLUSION

The Proposed RP-HPLC method is suitable for simultaneous determination of PQP and AM in Combined dosage form without any interferences from each other. The accuracy of the methods was assessed by recovery studies at three different levels. The method was found to be precise as indicated by the repeatability analysis, showing % RSD less than 2. All the parameters for both the drugs met the criteria of ICH guidelines for method validation. The developed method may be recommended for routine and QC analysis of the investigated drugs to provide simple, accurate and reproducible quantitative analysis for the determination of determination of PQP and AM in combined formulation.

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