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Development and validation of stability indicating RP-HPLC method for simultaneous estimation of Arterolane Maleate and Piperaquine Phosphate in pharmaceutical dosage form

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ABSTRACT

A simple, sensitive, robust and reproducible method for the simultaneous estimation of Arterolane maleate and Piperaquine phosphate in formulation was developed using Reverse phase high performance liquid chromatographic method. Reversed-phase chromatography was performed on Shimadzu Model SPD-20AT, using a mixture of phosphate buffer (pH-6.0): Methanol (80:20v/v) as a mobile phase with a flow rate of 1.0 ml/min. The analyte was examined with UV detector at 270 nm. In the developed method Arterolane maleate elutes at 3.687 min and Piperaquine phosphate at 5.880 min. The linearity of developed method was achieved in the range of 3.75-11.25 µg/ml for Arterolane maleate and 18.25-56.25 µg/ml for Piperaquine phosphate. The method was validated with respect to Linearity, Accuracy, Limit of Detection, Limit of Quantification, Robustness, System Suitability and Stability as per ICH guidelines. Arterolane maleate and Piperaquine phosphate were subjected to stress conditions including acidic, alkaline, oxidation, thermal and sunlight degradation. In these studies, 10.45% Arterolane maleate and 13.27% of Piperaquine phosphate were degraded in 0.1N HCl (1 hour). In 0.1 N NaOH (1 hour) 3.3% Arterolane maleate degradation and 25.38% Piperaquine phosphate degradation was observed. While treatment with 3% H_2O_2(1 hour) showed degradation of Arterolane maleate 15.41% and of Piperaquine phosphate 20.65%.Thermal exposure (half hour) showed 4.53% degradation of Arterolane maleate and 16.37% of Piperaquine phosphate. Sun light exposure (2 hour) showed 21.25% and 26.87% degradation of Arterolane maleate and Piperaquine phosphate respectively. Data revealed that developed method can be applied for routine quality control analysis for these drugs in pharmaceutical dosage form.

Key words: Arterolane maleate, Piperaquine phosphate, RP-HPLC, Force degradation.

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. AM 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].

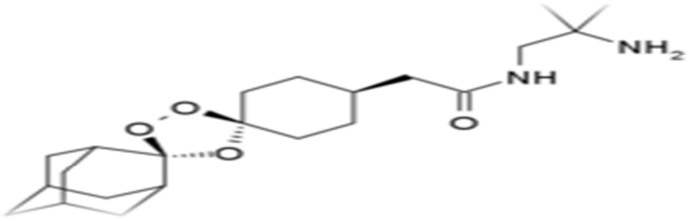

Fig. 1 Chemical Structure of Arterolane maleate

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].

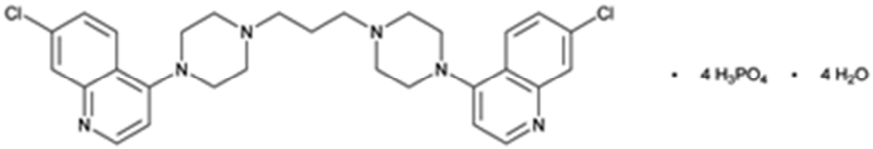

Fig. 2 Chemical Structure of Piperaquine phosphate

Analytical method development is defined as development, revision and application of validated, standardized and official methods of analysis. Method validation is the process of documenting or proving that selected method provides analytical data for the intended use. Method is validated by using parameters like accuracy, precision, linearity, limit of detection, limit of quantitation, system suitability, selectivity and specificity[4, 5]. The purpose of stability indicating method is to provide evidence on how the quality of a drug substance or product varies with time under the influence of a variety of environmental factor such as temperature, humidity and light and to establish retest period for the drug substance, or a shelf life for the drug product and recommended storage condition. According to ICH guidelines, in force degradation studies a variety of condition like pH, light, oxidation, dry heat etc. and separation of drug from degradation product is carried out [6, 7].

Combination of AM and PQP is available in tablet dosage form of 150:750 mg respectively. AM is official in Indian Pharmacopoeia 2014 [8]. PQP is official in United State Pharmacopoeia [3]. But combination of these drugs is not official in any pharmacopoeia. The combination of AM and PQP has been approved by Central Drug Standard Control Organization (CDSCO) on dated 19/10/2011 [9]. Very few methods like HPLC [10,11], Capillary zone electrophorosis[12], have been reported as a single or in combination with other drugs.

So, that need was felt, to develop new simple, accurate, precise stability indicating RP-HPLC method with good sensitivity for assay of AM and PQP in combined pharmaceutical dosage form using UV detection.

MATERIALS AND METHODS

Chemicals and Solvents

AM and PQP were procured from Gitar Laboratories, Ahmadabad, India as a gift sample. HPLC grade solvents: Water, Methanol, Acetonitrile were obtained from Merck India Ltd., Mumbai. SYNRIAM Tablet (Arterolane maleate 150 mg and Piperaquine phosphate 750 mg) was procured from local market.

HPLC instrumentation and chromatographic conditions

Separation and estimation was carried out using HPLC system a Hypersil BDS C_{18} 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.

A freshly prepared mixture of phosphate buffer (pH 6.0): methanol (80:20v/v) used as the mobile phase. Mobile phase was filtered through a 0.45µm membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1.0 ml/min.

Preparation of mobile phase

Mobile phase was prepared by mixing 800 ml phosphate buffer (pH 6) and 200 ml Methanol. Above mixture (80:20v/v) was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45µ filter under vacuum and sonicated for 10 min.

Preparation of Standard Solution

Accurately weighed 7.5 mg of standard AM and 37.5 mg of standard PQP and transferred to a 100 ml volumetric flask and dissolved in Methanol and sonicated for 15 minutes. 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 7.5µg/ml AM and 37.5µg/ml PQP.

Preparation of Sample solution

Twenty tablets were accurately weighed and ground to fine powder. Weigh and transferred tablet powder equivalent to AM 7.5 mg and PQP 37.5 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 up to mark with same solvent.

Selection of analytical wavelength

The standard solution of AM and PQP were scanned in the UV region of 200-400 nm using methanol as a blank and the overlain spectra was recorded. 270 nm analytical wavelength was selected for estimation of AM and PQP. (Fig. 3)

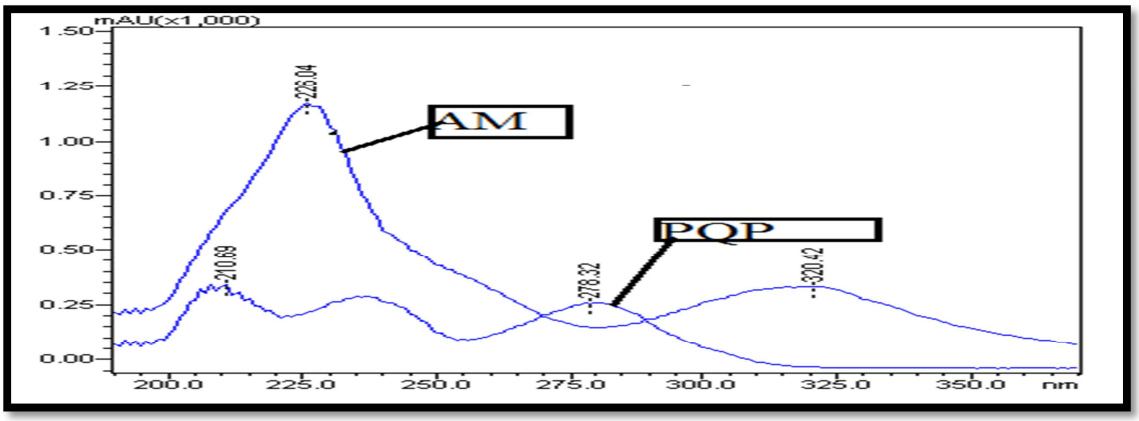

Fig. 3 Selection of detection wavelength (270nm)

Optimization of HPLC method

The pure drug solution of AM and PQP were injected individually into HPLC system and allow to run in different mobile phases like methanol, water, acetonitrile and phosphate buffer in different proposition to find the optimum conditions for the separation of AM and PQP. It was found that mobile phase containing phosphate buffer (pH 6.0): methanol (80:20v/v) at a flow rate of 1.0 ml/min with detection wavelength 270nm gave satisfactory results with sharp, well defined and resolved peaks with minimum tailing as compared to other mobile phase. Under this conditions the retention time were typically 3.687 min for AM and 5.880 min for PQP (fig. 4)

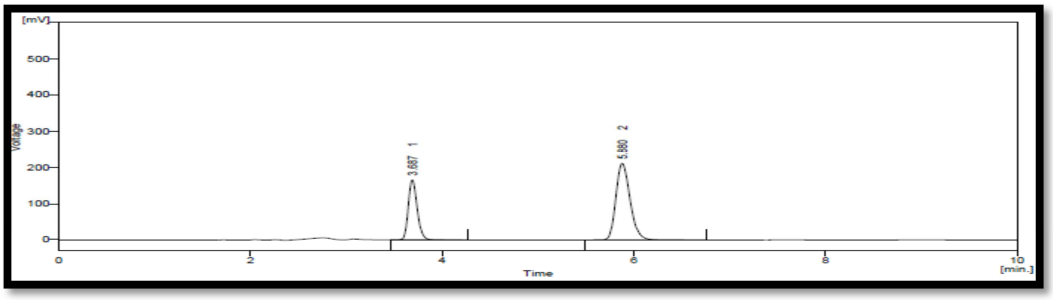

Fig. 4 Chromatogram of AM and PQP for optimized method

Method Validation
The developed method was validated as per ICH guidelines for following parameters:

Linearity, Precision, Accuracy, Sensitivity, Robustness.

Forced degradation studies
The study was intended to ensure the effectiveness preparation of AM, PQP and its degradation peaks of formulation ingredients at the retention time of AM and PQP. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the methods.

Acid degradation
Forced degradation in acidic media was performed by keeping the standard solution in contact with 0.1 N HCl for 1 hr at room temperature. After 1 hr, the solution was neutralized with 0.1 N NaOH and diluted up to 10 ml with mobile phase. Dilution was done to achieve the appropriate concentration 7.5 µg/ml of AM and 37.5 µg/ml of PQP. Result is shown in (fig. 5)

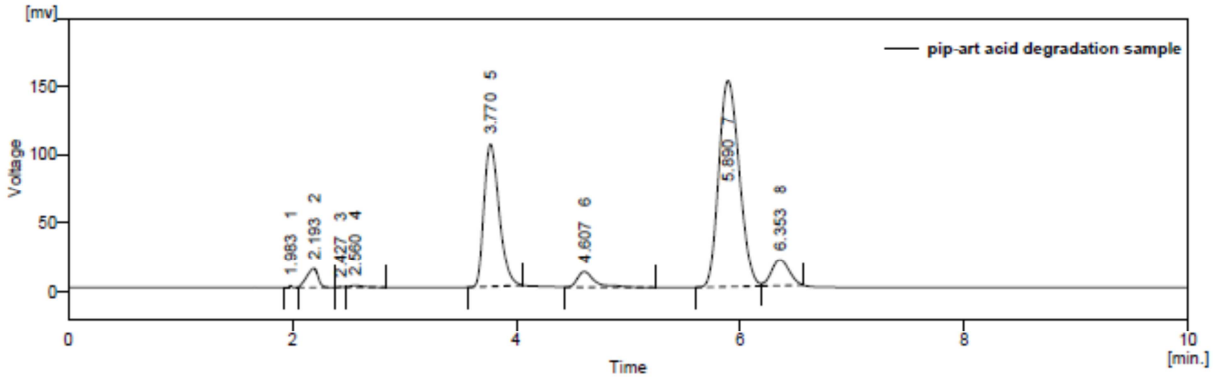

Fig. 5 Chromatogram of combined AM and PQP mixture in acid degradation

Basic degradation
Forced degradation in basic media was performed by keeping the standard solution in contact with 0.1 N NaOH for 1 hr at room temperature. After 1 hr, the solution was neutralized with 0.1 N HCl and diluted up to 10 ml with mobile phase. Dilution was done to achieve the appropriate concentration 7.5 µg/ml of AM and 37.5 µg/ml of PQP. Result is shown in (fig. 6)

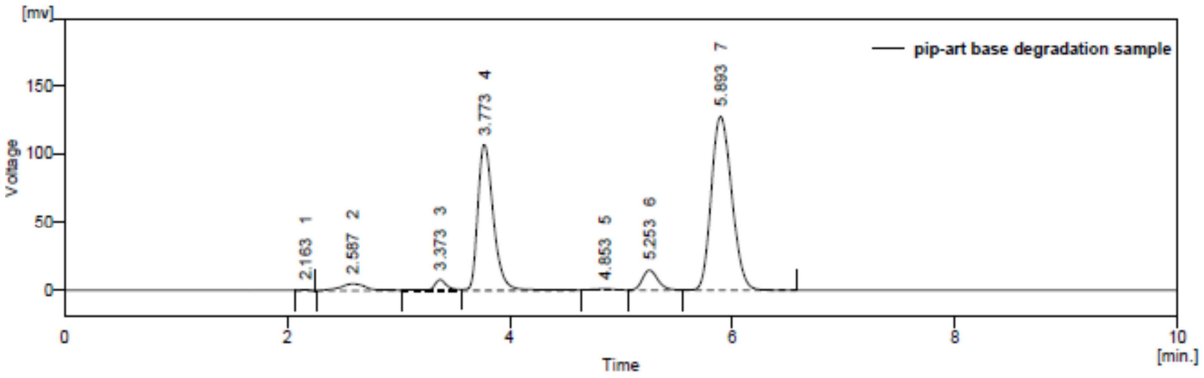

Fig. 6 Chromatogram of combined AM and PQP mixture in base degradation

Oxidation degradation

Forced degradation in 3% H_2O_2 media was performed by keeping the standard solution in contact with 3% H_2O_2 for 1 hr at room temperature. After 1 hr, the solution was diluted with mobile phase up to 10 ml to achieve the appropriate concentration 7.5 µg/ml of AM and 37.5 µg/ml of PQP. Result is shown in (fig. 7)

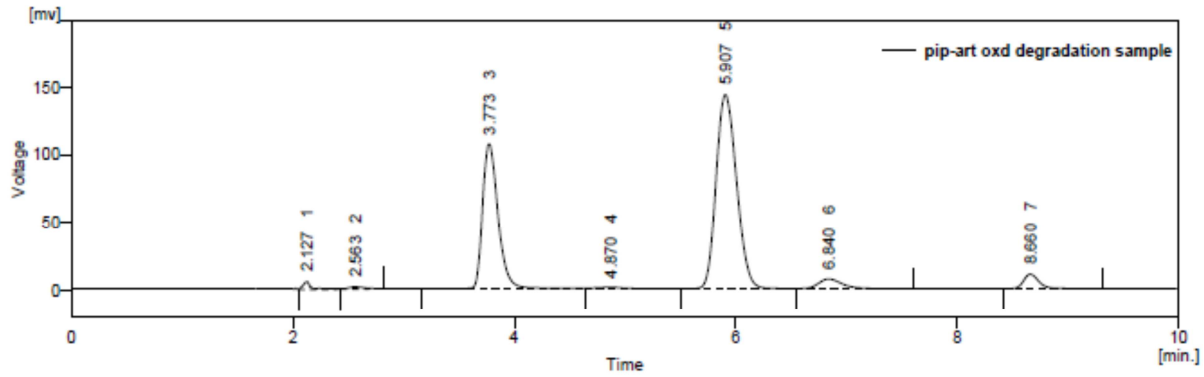

Fig. 7 Chromatogram of combined AM and PQP mixture in oxidative degradation

Thermal degradation

Sample solution was exposed to temperature of 105ºC for 30 min in an oven. After 30 min, solution was diluted with mobile phase up to 10 ml to achieve the appropriate concentration 7.5 µg/ml of AM and 37.5 µg/ml of PQP. Result is shown in (fig. 8)

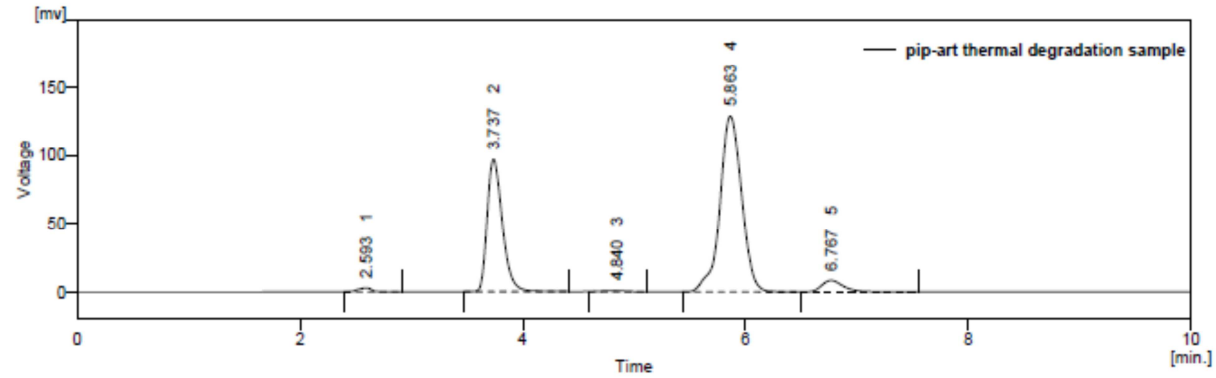

Fig. 8 Chromatogram of combined AM and PQP mixture in thermal degradation

Sunlight degradation

Sample solution was exposed in the sunlight for 2 hrs. After 2 hrs solution was diluted with mobile phase up to 10 ml to achieve the appropriate concentration 7.5µg/ml of AM and 37.5 µg/ml of PQP. Result is shown in (fig. 9)

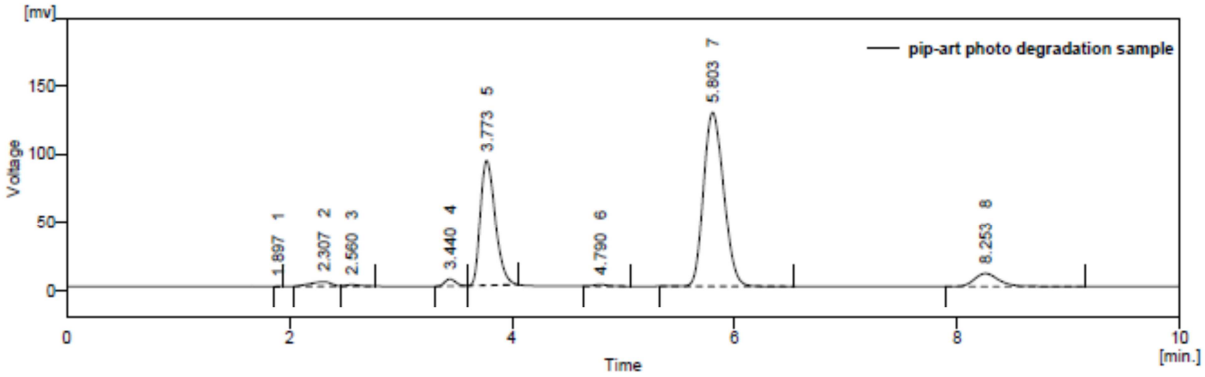

Fig. 9 Chromatogram of combined AM and PQP mixture in sunlight degradation

RESULTS AND DISCUSSION

Linearity

The drug response was linear (r^2 = 0.999 for AM and 0.997 for PQP) over the concentration range between 3.75-11.25 µg/ml for AM and 18.25-56.25 µg/ml for PQP. The result is shown in (Table 1)

Table 1 linearity data of AM and PQP

Parameters	AM	PQP
Concentration Range (µg/ml)	3.75-11.25	18.25-56.25
Regression equation (y= mx + c)	y = 147.4x-11.78	y = 62.10x-91
Slope (m)	147.4	62.10
Intercept (c)	11.78	91
Correlation Coefficient ($r^{2)}$	0.999	0.997
LOD(µg/ml)	0.26	2.791
LOQ(µg/ml)	0.79	8.45

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 0.26µg/ml and 0.79µg/ml for AM and 2.791µg/ml and 8.45µg/ml for PQP respectively (Table-1).

Precision

The results of the repeatability, intra-day and inter-day precision experiments are shown in Table 2, 3. 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.

Table 2 Repeatability study of AM and PQP

Concentration	AM (7.5 µg/ml)	PQP (37.5 µg/ml)
Area* (NMT-2%)	1100.136	2228.045
± SD	6.780055145	8.853183
%RSD	0.61629236	0.397352

**Average of six determinations, SD - standard deviation and RSD-Relative standard deviation*

Table 3 Intra-day and Inter-day precision of AM and PQP

Drug	Concentration (µg/ml)	Intra-day area* ± SD	%RSD	Inter-day area* ± SD	%RSD
AM	3.75	543.372±3.162	0.5820323	541.70±4.563	0.8423
	7.5	1095.328±8.590	0.7843093	1096.10±4.374	0.3990
	11.25	1640.11±14.030	0.8554900	1640.10±12.398	0.7559
PQP	18.75	1094.36±13.228	1.2087535	1092.54±13.228	1.2107
	37.5	2211.74±13.897	0.6283396	2212.05±16.045	0.7253
	56.25	3323.95±31.454	0.9462924	3322.88±29.431	0.8857

**Average of three determinations*

Accuracy

Recovery studies:

As shown in Table-4,5 good recoveries of the AM and PQP in the range from 98% to 102 % were obtained at various added concentrations.

Table 4 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
80	3.75	3	6.75	475.525	2.99	99.66
100	3.75	3.75	7.5	586.147	3.73	99.69
120	3.75	4.5	8.25	702.904	4.48	99.63

**Average of three determination*

Table 5 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
80	18.75	15	33.75	873.46	14.96	99.79
100	18.75	18.75	37.5	1096.60	18.72	99.84
120	18.75	22.5	41.25	1316.09	22.44	99.73

**Average of three determinations, Recovery should be 98-102%*

Robustness:

The standard deviation of the peak areas was calculated for each parameter and the %RSD was found to be less than 2 %. Results shows low values of % RSD, as shown in Table 6 signify the robustness of the method.

Table 6 Robustness data of AM and PQP

Parameters	Normal Condition	Change in Condition	Drug	Area* ± SD	% RSD	Retention time (min)	Theoretical plates
Mobile phase ratio (phosphate buffer: methanol (80:20w/v) (±2.0)	80:20	78:22	AM	1128.949±9.09	0.8059	3.82	6966
			PQP	2275.71±30.76	1.351	6.09	7110
		82:18	AM	1068.31±21.08	1.974	3.63	7011
			PQP	2170.07±22.58	1.040	5.79	7270
Change in flow rate (±0.2)	1.0 ml/min	0.8 ml/min	AM	1141.90±9.39	0.822	3.86	6962
			PQP	2306.06±21.45	0.930	6.15	7105
		1.2 ml/min	AM	1075.56±11.48	1.067	3.61	7011
			PQP	2171.58±24.30	1.119	5.76	7263
Change in pH (±0.2)	6.0	5.8	AM	1133.11±7.91	0.698	3.82	6966
			PQP	2282.92±25.78	1.129	6.09	7110
		6.2	AM	1050.39±16.60	1.580	3.56	7025
			PQP	2129.98±21.64	1.01	5.67	7268

**Average of three determinations, SD- standard deviation*

Forced Degradation study

Results for stress degradation studies of AM and PQP are shown in the table 7 and 8 respectively. The results of the methods lie within the prescribed limit, showing that method is free from interference from excipient.

Table 7 Results of forced degradation study of AM

Stress conditions	Time (min)	Retention time(min)	Area	%Area	Degradants (% area)
0.1 N HCl	60	3.770	973.228	89.55	10.45
0.1 N NaOH	60	3.773	1049.776	96.70	3.3
3% H_2O_2	60	3.773	919.248	84.59	15.41
Heat exposure	30	3.73	1037.557	95.47	4.53
Sunlight	60 120	3.68 3.77	1086.686 855.85	100 78.75	Absent 21.25

Table 8 Results of forced degradation study of PQP

Stress conditions	Time (min)	Retention time(min)	Area	%Area	Degradants (% area)
0.1 N HCl	60	5.890	1907.76	86.73	13.27
0.1 N NaOH	60	5.893	1641.279	74.62	25.38
3% H_2O_2	60	5.907	1745.414	79.35	20.65
Heat exposure	30	5.863	1839.518	83.63	16.37
Sunlight	60 120	5.880 5.803	2199.478 1608.572	100 73.13	Absent 26.87

CONCLUSION

The proposed stability- indicating RP-HPLC method is suitable for simultaneous estimation of AM and PQP in pharmaceutical dosage form without any interferences from each other. All the parameters for both the drugs met the criteria of ICH guidelines for method validation. The results show that the developed method was accurate, precise, simple, specific,robust and found to be stability indicating under stress conditions.

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