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SIMULTANEOUS ESTIMATION OF ARTEROLANE MALEATE AND PIPERAQUINE PHOSPHATE BY RP-HPLC

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ABSTRACT

A quick and reproducible superior fluid chromatography technique was created and approved for the estimation of Arterolane Maleate, Piperaquine phosphate in dosage forms. Chromatography is done isocratically on Xbridge C18 (100nm X 4.6nm X 5µm) with a 0.1% Ortho Phosphoric Acid and Acetonitrile (in the proportion of 90:10 (v/v) at a flow of 0.9 ml/min. Measurement was done utilizing a PDA locator at 290nm. The separation time of Arterolane Maleate (ART) and Piperaquine Phosphate (PQP) are 2.9, 6.9 min individually. The Linearity of the method extend and % recovery of Arterolane and Piperaquine are 20-150µg/ml and 99%, 101% separately. The regression of ART and PQP are 0.999. The % relative standard deviations for three reproduce estimations of tests in tablets are under 2%. Developed technique was seen as rapid for synchronous estimation of Arterolane Maleate and Piperaquine phosphate in Pharmaceutical dose formulations. The proposed strategy can be helpful in quality control of bulk manufacturing and Pharmaceutical formulations.

KEYWORDS

Arterolane Maleate, Piperaquine phosphate, HPLC, Validation and Retention time.

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INTRODUCTON

Arterolane maleate (Figure No.1) is chemically cis-admantane-2-spiro-3'-8'-[[[(2'- amino-2'methyl Propyl) amino] carbonyl] methyl] 1'2'4'-trioxaspiro [4, 5] decane hydrogen maleate. Arterolane maleateis a Synthetic peroxide which acts as anti-malarial agent by rapidly acting as blood schizonticide against all blood stages of P. *falciparum* without having effect on liver stages[1].

Piperaquine phosphate (Figure No.2) is chemically 1, 3- Bis (4-(7-chloroquinolin-4-yl) piperazin-1-yl) propane tetraphosphate tetrahydrate[2].

Arterolane maleate and Piperaquine phosphate in

combination are available in tablet dosage forms in the ratio of 150: 750. Literature survey reveals Automated Solid Phase Extraction and Liquid Chromatographic Method[3], Capillary Zone Electrophoresis[4], HPLC[5] and LC/MS/MS[4,6-11] methods for the estimation of Piperaquine phosphate alone or in combination with other drugs in pharmaceutical formulation and biological samples wherea s headspace gas chromatographic[12] methods for the estimation of Arterolane maleate alone in pharmaceutical formulation and biological samples. There are very few reported methods for the simultaneous determination of Arterolane maleate and Piperaquine phosphate by RP-HPLC in pharmaceutical dosage forms[13,14].

The apparent lack of simple method for the estimation of Arterolane maleate and Piperaquine phosphate by RP-HPLC in pharmaceutical dosage forms prompted us to develop this method. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines[15-18].

MATERIAL AND METHODS

Instrumentation

Chromatographic separation changed into achieved on a agilent chromatographic machine prepared with 1200 collection isocratic pump; rheodyne injector with 20µl fixed extent loop, variable wavelength programmable UV detector and the output sign changed into monitored and incorporated through ezichrome elite chromatographic software. Double beam UV-VIS spectrophotometer (labindia-3120) was used to carry out spectral evaluation and the information was recorded by uvwin-5 software. Ultrasonicator (1. 5l) became used for degasification of samples. Sample drugs had been weighed through using shimadzu digital analytical balance (ax-220) and pH was adjusted by means of the use of systronics digital pH meter.

Chemical compounds and solvents

All chemicals and reagents used were of HPLC grade. Arterolane maleate and piperaquine procured from micro labs, Bangalore. The other reagents used were methanol and acetonitrile from qualigens ltd. Mumbai, India, orthophosphoric acid from hi-media, Mumbai, India, and HPLC grade water from merck chemical, Mumbai, India.

Chromatographic conditions

Separation and estimation was done using HPLC (waters-2469 with PDA detector), column utilized in experiment with C18 waters hypersil ODS, 5µ (150 x 4.6mm). The mobile phase become prepared with the aid of blending 0.01m potassium dihydrogen phosphate buffer: methanol (ph-2. 6) in the ratio of (60: forty) was filtered and degassed. Injection extent is 10µl and the measurement was at 240nm.

Instrumentation

Chromatographic separation was performed on a Agilent chromatographic system equipped with 1200 series isocratic pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector and the output signal was monitored and integrated by EZICHROME ELITE Chromatographic Software. Double beam UV-Visible spectrophotometer (Labindia-3120) was used to carry out spectral analysis and the data was recorded by UVWIN-5 software. Ultrasonicator (1.5L) was used for degasification of mobile phase and samples. Standard and sample drugs were weighed by using shimadzu electronic analytical balance (AX-220) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Chemicals and solvents

All chemicals and reagents used were of HPLC grade. Pure standards of Arterolane Maleate and Piperaquine employed in the study were obtained as gift sample from MICRO LABS, Bangalore. The other reagents used were Methanol and Acetonitrile from Qualigens ltd. Mumbai, India, Ortho Phosphoric Acid from Hi-media, Mumbai, India, and HPLC grade water from Merck chemicals, Mumbai, India.

Chromatographic conditions

Separation and estimation was carried out using HPLC (waters-2469 with PDA detector), column used in experiment was C18 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 measurement was at 240nm.

Preparation of standard stock solution

Standard stock solution of Arterolane Maleate and Piperaquine pure drug (1mg/ml) was prepared by precisely weighing about 100mg drug and transferring in to 100ml volumetric flask and dissolved in diluent.

Preparation of Buffer (0.1% Ortho Phosphoric Acid- pH adjusted to 2.5)

Transferred 1ml of Concentrated Ortho phosphoric acid in a 1000ml of Volumetric flask add about 900ml of milli-Q water added add 1ml of triethylamine and degas in ultrasonic water bath for 10 minutes and finally make up the volume with water, then pH adjusted to 2.5 with dil. Ortho phosphoric acid solution. Filter through 0.45µ filtered under vacuum filtration.

Preparation of mobile phase

The mobile phase used was Acetonitrile and freshly prepared 0.1% Ortho Phosphoric Acid buffer solution (P^H 2.5) in the ratio of 90: 10 (v/v) and the mobile phase was filtered through 0.45µ membrane filter and sonicated before use.

Preparation of Diluent

Transfer measured volume of 50ml methanol in 100ml volumetric flask and add 50ml of milli-Q water. Filtered through 0.45µ membrane filter and sonicated before use.

METHOD VALIDATION

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.

System suitability of the method

Standard concentration 20µl of the sample was injected into HPLC system and the results obtained were used to express the system suitability of the developed method. The results were depicted in Table No.1.

Linearity of the method and Range

A series of standard concentrations were prepared from 50% to 150% of the target concentration of ALM and PQP. Linearity of the method of ALM was found to be exist between 30-225µg/ml and for PQP was 50-1125µg/ml. The chromatograms were recorded and Linearity of the method graph was plotted by using peak area of drug against respective concentrations to obtain the Linearity of the method range. The results were depicted in Table No.2 and Figure No.3.

Precision of the method

The intra-day and inter-day Precision of the method studies were carried out using a test sample assay method with six replicates on the same day and different days. The results were depicted in Table No.3 to Table No.4.

Accuracy (Recovery)

The accuracy of the method was determined by calculating recoveries of ALM and PQP by method of standard additions. Known amount of ALM and PQP were added to a pre quantified sample solution (containing ALM and PQP in 100µg/ml proportion, respectively). The results were depicted in Table No.5 to Table No.6.

Ruggedness

Ruggedness was carried out by using a test sample assay method with six replicates using different analyst, column and system. The results were depicted in Table No.7.

Robustness

Robustness was carried out by small variation in the chromatographic conditions at a concentration equal to standard concentrations 100µg/ml for ALM and 100µg/ml for PQP and %change was calculated. % change in the results was calculated. The results were depicted in Table No.8.

Detection limit and Quantification limit

LOD and LOQ were calculated using the following equation as per ICH guidelines. LOD = 3 .3 × σ / S; L OQ = 1 0 × σ / S.

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve. The results were depicted in Table No.9.

Solution Stability

Solution stability was assed using standard and test

stock solutions. These stocks were prepared and stored at room temperature and refrigerated conditions (2-8°C) for 36 hrs and % differences was calculated. The results were depicted in Table No.10 to Table No.13.

Filter validation

Portion of the test solution was filtered through three different filters namely 0.45µm PVDF filter, 0.45µm PTFE and 0.45µm Nylon filter and some portion was centrifuged and injected into the HPLC system. The % difference values between centrifuged and filtered sample were calculated. The results were depicted in Table No.14.

Preparation test solution (ANALYSIS OF MARKETED FORMULATION)

Twenty test tablets have been weighed and fine powdered. An amount of powder equivalent to 150mg of arterolane and 750mg of piperaquine (synriam pills) were weighed accurately and transferred right into a hundred ml volumetric flask containing 25ml of water: methanol (50: 50) and sonicated for 20 min with intermediate shaking for whole extraction of drugs and diluted to 100ml with water: methanol (50: 50), then filtered through 0.45µm membrane to clear out and 5ml of filtrate taken into 50ml volumetric flask and made as much as the quantity with water : methanol (50: 50) and injected in to HPLC. The results had been depicted in Table No.15 and Figure No.4.

RESULTS AND DISCUSSION

On this RP-HPLC approach, the conditions had been optimized to attain an ok separation of eluted compounds. To start with, various mobile phase compositions have been tried, to separate analytes. The column segment and flow rate choice changed into based totally on parameters (top, tailing, theoretical plates, ability or symmetry element), run time and determination. The system with 0.1% ortho phosphoric acid buffer: acetonitrile (90: 10v/v) at drift rate flown of 1.0ml/min changed into determined to be robust technique. The evolved technique became tested as in line with the ICH guidelines for the quantification of arterolane and piperaquine in pharmaceutical formulations. A suitability test applied to and the results received were inside desirable limits of tailing issue ≤2.0 and theoretical plates >2000. The calibration curve turned into constructed with series of standards of 30-225µg/ml and 50-1125µg/ml for arterolane and piperaquine. This concluded that the approach become linear and accurate. Specificity become studied for the quantification of excipients inside the pill dosage form of arterolane and piperaquine. From the consequences it changed into indicated that none of excipients have been intrude at analytes retention time. The precision of the technique turned into measured in terms of repeatability, which turned into decided with the aid of sufficient wide variety of aliquots of a homogenous sample with inside the day (intraday) and next consequent 3 days for inter day Precision of the method. For every instances % RSD became calculated and outcomes had been the perfect limits. The low values of RSD imply that the method changed into precise. . The % recovery for each case become calculated and observed to be 99.98 to 100.18% for ALM and 99.51 to 100.09 for PQP and observed to be consequences had been within recognition limits. Therefore the developed approach is accurate in the course of the chosen variety published methods. Robustness check became accomplished via small variant inside the chromatographic situations and % change was calculated. The % change inside the results was calculated and results found to be below 2.0%. A signal-to-noise ratio 2: 1 is typically taken into consideration for estimating the detection limit. LOD is determined to be 2.50034µg/ml for ALM and 7.5767g/ml for PQP and LOQ is found to be 2.94827µg/ml for ALM and 8.934µg/ml for PQP. Sample are stable at 5°C for 36 hrs because the % difference was less than 2.0%. Filter out interference was carried out on three sorts of 0.45µ filters (nylon, pvdf) and the % difference found to be underneath 2.0%. The demonstrated approach was carried out for the assay of marketed pills of ALM AND PQP (synriam capsules). The % assay determined to be 101.5% for ALM and 100.2% for PQP.

Table No.1: System Precision of Arterolanemaleate and piperaquine Phosphate

Retention Time	Arterolane	2.97 min
	Piperaquine	6.97 min
Peak Area	Arterolane	1963213
	Piperaquine	8824977
Theoretical plates	Arterolane	3699
	Piperaquine	5430
Tailing Factor	Arterolane	1.4
	Piperaquine	1.32
Resolution	Arterolane	-
	Piperaquine	5.9

Table No.2: Linearity of the method and range of ALM and PQP

S.No	Concentration µg/ml	Area of ALM	Concentration µg/ml	Area of PQP
1	30	386185	50	956891
2	75	960922	75	1105678
3	105	1355242	525	6040731
4	150	1963213	750	8824977
5	180	2357969	900	10595121
6	225	2942729	1125	13242269
Concentration range		30-225µg/ml	150-11125ml	
Slope (m)		150036	18956	
Correlation coefficient		0.9975	0.9782	

Table No.3: Intraday Precision of the method data for ALM and PQP

Sample. No	Area of ALM	% Label Claim	Area of PQP	% Label Claim
1	1440866	100.39	6484965	99.25
2	1428777	99.55	6594345	100.92
3	1430407	99.66	6560373	100.40
4	1423047	99.15	6576012	100.64
5	1421048	99.01	6524590	99.86
6	1442223	100.49	6501156	99.50
Mean	1431061	99.71	6540240	100.096
SD	8841.45	0.616	43451.43	0.665
%RSD	0.617	0.617	0.664	0.664

Table No.4: Inter-day Precision of the method data for ALM and PQP

Sample. No	Area of ALM	% Label Claim	Area of PQP	% Label Claim
1	1416000	99	6987622	99.50
2	1463054	100.45	6906570	99.22
3	1494373	99.34	6729675	100.17
4	1437579	99.98	6762279	99.36
5	1459677	100.07	6873276	100.03
6	1458394	99.21	6729367	100.67
Mean	1454846	99.90	6814798	99.96
SD	26354.6	0.99	107067.2	0.5742
%RSD	1.80	1.30	1.50	0.9742

Table No.5: Accuracy of ALM

S.No	Level of % recovery	Target Conc. (µg/ml)	Amount of drug Spiked (µg/ml)	Amount found (µg/ml)	%Recovery	Mean	SD	%RSD
1	80	100	80	3.015	100.5	101.22	0.048	1.51
				3.061	98.80			
				2.965	102.79			
2	100	100	100	4.052	101.3	100.34	0.0482	1.28
				3.956	98.90			
				4.013	100.32			
3	120	100	120	5.016	100.32	101.65	0.0684	1.36
				5.103	102.06			
				4.968	99.03			

Table No.6: Accuracy of PQP

S.No	Level of % recovery	Target Conc. (µg/ml)	Amount of drug spiked (µg/ml)	Amount found (µg/ml)	%Recovery	Mean	SD	%RSD
1	80	200	160	360.45	99.34	99.67	0.4490	1.06
				360.23	99.12			
				360.14	99.78			
2	100	200	100	300.23	100.80	99.00	0.7862	1.77
				300.43	99.10			
				300.56	99.25			
3	120	200	240	440.32	100.27	100.13	0.546	1.54
				440.18	99.50			
				440.97	99.85			

Table No.7: Ruggedness of ALM and PQP

S.No	ALM (%Assay)			PQP (%Assay)		
	SET I	SET II	SET III	SET I	SET II	SET III
1	99.43	99.83	99.43	99.03	99.67	99.67
2	100.91	100.01	100.91	100.41	100.01	100.61
3	98.64	98.24	98.64	98.74	98.34	98.88
4	103.56	103.66	103.56	103.06	103.76	103.03
5	100.58	100.58	100.58	100.28	100.54	100.43
6	102.67	102.07	102.67	102.07	102.12	102.67
Average	100.80	100.10	100.80	100.40	100.23	100.37
SD	1.21	1.45	1.914	1.344	1.614	1.34
% RSD	1.4	1.2	1.6	1.56	1.53	1.33
Overall Average	101.9			100.71		
Overall % RSD	1.05			1.05		

SET - I: Variability due to HPLC system
SET - II: Variability due to HPLC column
SET - III: Variability due to analyst

Table No.8: Robustness of CFX and AZT

S.No	Parameter	Condition	ALM		PQP	
			Area (n=3)	% change	Area (n=3)	% change
1	Standard	Standard conditions	1438318	0	6529991	0
2	Mobile phase	0.1% Ortho Phosphoric acid (pH-2.5): Acetonitrile	1414243	0.1	6527790	0.14
		0.1% Ortho Phosphoric acid (pH-2.5): Acetonitrile	1446992	1.33	6536602	0.55
3	Mobile phase pH	2.7	1432336	0.83	6526608	0.11
		2.3	1423680	0.52	6553068	0.56
4	Wavelength	288nm	1446791	0.19	6490488	0.07
		292nm	1438318	0.23	6527425	1.51
5	Flow rate	1.2	1414243	0.25	6490465	0.07
		0.8	1446992	0.18	6527234	1.51

Table No.9: LOD and LOQ of ALM and PQP

S.No	Parameter	ALM	PQP
1	LOD(µg/ml)	2.50034	7.5767
2	LOQ(µg/ml)	2.94827	8.9341

Table No.10: Solution Stability of ALM at room temperature

S.No	Time	Standard stock			Test stock		
		Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
1	Initial	1438318	1438543	**NA**	1438324	1438312	**NA**
2	6hrs	1414243	1414240	0.1	1414268	1414245	0.7
3	12hrs	1446992	1446981	0.0	1446911	1446993	0.9
4	20hrs	1432336	1432333	0.3	1432362	1432339	1.2
5	26hrs	1423680	1423626	0.8	1423682	1423681	0.1
6	30hrs	1446791	1446791	0.2	1446797	1446795	0.4
7	36hrs	1433727	1433761	0.5	1433729	1433721	0.8

Table No.11: Solution Stability of PQP at room temperature

S.No	Time	Standard stock			Test stock		
		Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
1	Initial	6529991	6529992	NA	6529993	6529990	NA
2	6hrs	6527790	6527793	0.1	6527795	6527793	0.7
3	12hrs	6536602	6536645	0.0	6536601	6536605	0.9
4	20hrs	6526608	6526662	0.3	6526607	6526601	1.2
5	26hrs	6553068	6553061	0.8	6553068	6553067	0.1
6	30hrs	6490488	6490482	0.2	6490481	6490489	0.4
7	36hrs	6527425	6527421	0.5	6527424	6527421	0.8

Table No.12: Solution Stability of ALM at refrigerated temperature

S.No	Time	Standard stock			Test stock		
		Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
1	Initial	1438312	1438318	NA	1438318	1438311	NA
2	6hrs	1414241	1414243	0.1	1414243	1414247	0.7
3	12hrs	1446993	1446990	0.0	1446991	1446996	0.9
4	20hrs	1432335	1432336	0.3	1432356	1432332	1.2
5	26hrs	1423686	1423680	0.8	1423620	1423685	0.1
6	30hrs	1446792	1446791	0.2	1446701	1446790	0.4
7	36hrs	1433723	1433727	0.5	1433722	1433722	0.8

Table No.13: Solution Stability of PQP at refrigerated temperature

S.No	Time	Standard stock			Test stock		
		Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
1	Initial	6529991	6529993	**NA**	6529909	6529993	**NA**
2	6hrs	6527790	6527795	0.1	6527792	6527791	0.7
3	12hrs	6536602	6536602	0.0	6536601	6536600	0.9
4	20hrs	6526608	6526609	0.3	6526608	6526604	1.2
5	26hrs	6553068	6553060	0.8	6553062	6553012	0.1
6	30hrs	6490488	6490484	0.2	6490486	6490432	0.4
7	36hrs	6527425	6527422	0.5	6527423	6527421	0.8

Table No.14: Filter Interference Results for ALM and PQP

S.No	ALM				
	Filtration Method	**Centrifuged**	**Nylon**	**PTFE**	**PVDF**
1	Area (Inj. 1)	1438318	1438313	1438312	1438319
2	Area (Inj. 2)	1414243	1414244	1414241	1414242
3	Avg. Area	1446992	1446995	1446996	1446992
4	% Difference		-0.2	0.2	0.5
	PQP				
	Filtration Method	**Centrifuged**	**Nylon**	**PTFE**	**PVDF**
5	Area (Inj. 1)	6529990	6529990	6529994	6529991
6	Area (Inj. 2)	6527792	6527791	6527795	6527790
7	Avg. Area	6536603	6536602	6536602	6536602
8	% Difference		-0.4	0.3	0.5

Table No.15: Analysis of Commercial Formulation

S.No	Tablet	Label claimed(mg)		Conc.found (mg)		% Assay (n=6)	
1	SYNRIAM *Tablets*	ALM	PQP	ALM	PQP	ALM	PQP
		150	750	15.02	1.51	101.5	100.2

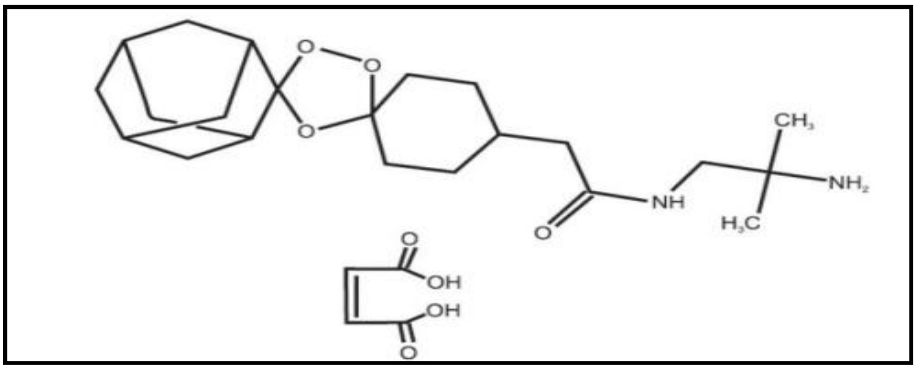


Figure No.1: Structure of arterolane maleate

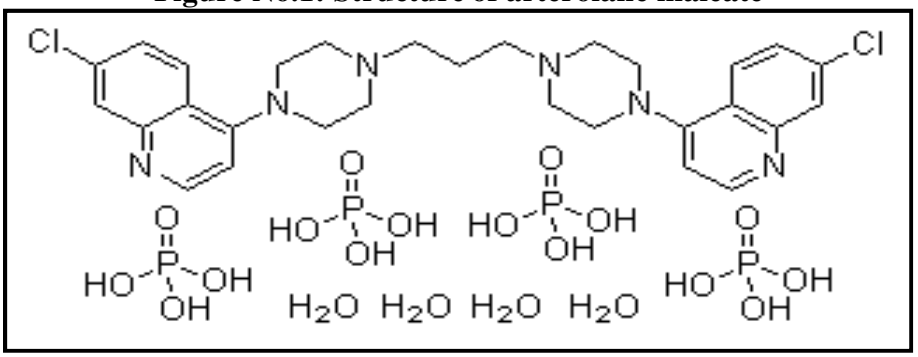
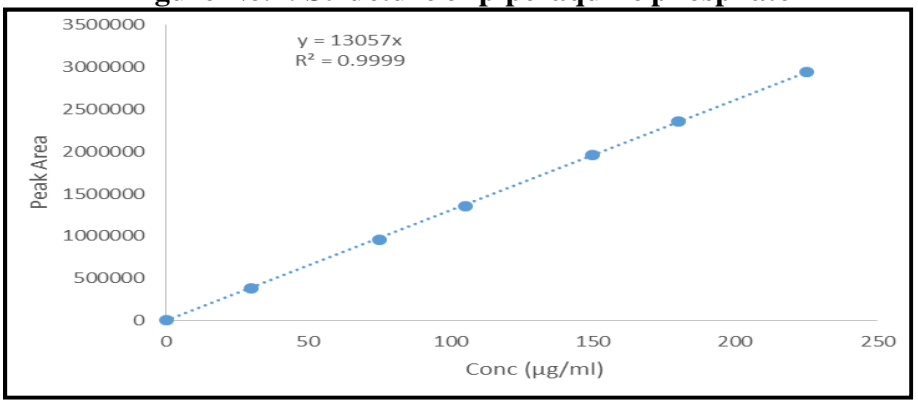


Figure No.2: Structure of piperaquine phosphate



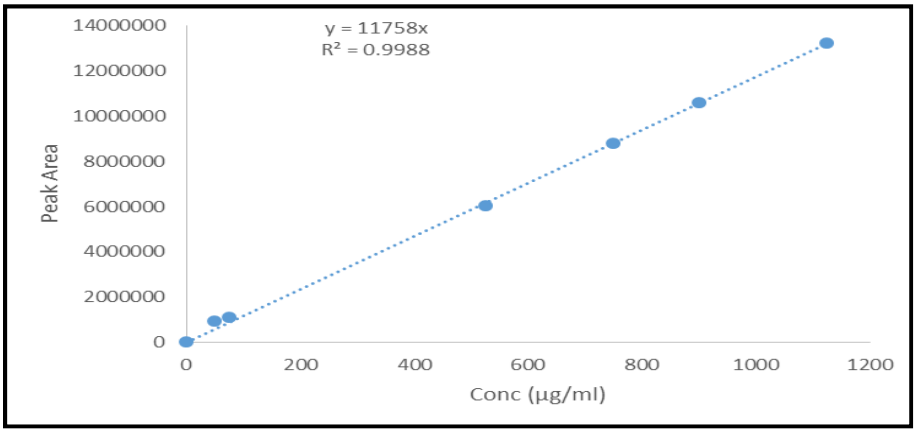


Figure No.3: Linearity of the method of ALM and PQP

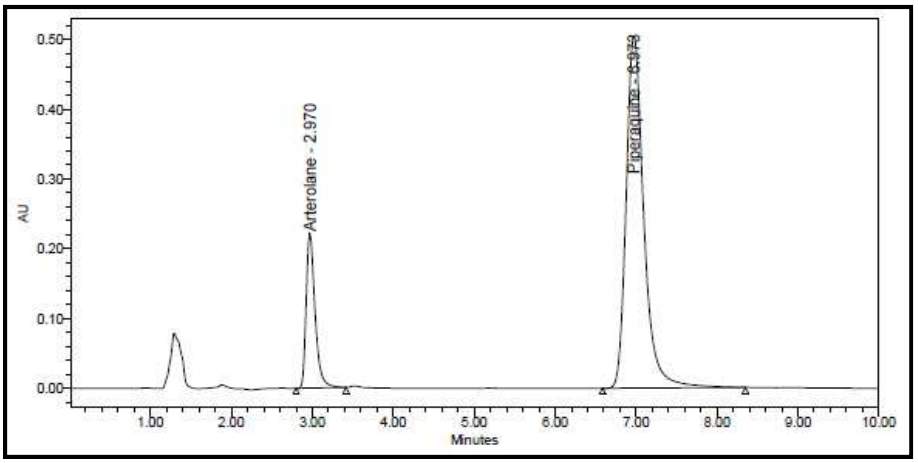


Figure No.4: Test formulation chromatogram of Arterolane and Piperaquine

CONCLUSION

Thus the method developed in the present investigation is simple, selective and rugged. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. Hence, the developed method can be successfully applied for the estimation of Arterolane and Piperaquine in tablet dosage forms by RP-HPLC.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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