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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PIPERAQUINE PHOSPHATE AND DIHYDROARTEMISININ IN BULK

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ABSTRACT

High Performance Liquid Chromatography (HPLC) methods are described for determination of drugs as a single or in combination in bulk or pharmaceutical formulation. The objective of the present study was to develop and validate novel, accurate, sensitive, precise, rapid and isocratic reverse Phase HPLC (RP-HPLC) method for the simultaneous determination of Piperaquine phosphate and Dihydroartemisinin in bulk because no method is available for simultaneous estimation of these drugs. The separation was achieved on GRACESMART RP-18 column (250 mm × 4.6 mm, 5µm) with mobile phase consisting of 10 mM Ammonium acetate (pH4.6, adjusted with Acetic acid): Methanol (15:85 % v/v) at a flow rate of 1.2 ml/min. UV detection at 220 nm. PQP and DHA obeyed linearity in the concentration range of 5-25 µg/ml (r2 = 0.9993) and 5-25 µg/ml (r2 = 0.9987) respectively. The asymmetric factors were found to be 1.17 for PQP and 1.2 for DHA. The developed method was validated as per ICH guidelines fulfill all the acceptance criteria and can be use for routine analysis.

Keywords: Piperaquine phosphate, Dihydroartemisinin, Simultaneous, RP-HPLC.

INTRODUCTION

Piperaquine Phosphate (PQP) i.e. 4,4-(1,3-Propanediyldi-4,1-Piperazinediyl) bis (7- chloroquinoline) phosphate, molecular mass is 633.51 g/mole 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.

Dihydroartemisinin (DHA) is (4S,5R,8S,9R,10S,12R,13R)-1,5,9-Trimethyl 11,14,15,16- tetraoxatetracyclo [10.3.1.0[4,13].0[8,13]] hexadecan-10-ol. DHA is Dihydroartemisinin (DHA) is a semisynthetic compound obtained by reduction of artemisinin extracted from the leaves of *Artemisia annua* L followed by purification and blending. DHA is highly active against asexual forms of the four species of Plasmodium that infect humans with very rapid reductions in parasitaemia but relatively short plasma half-lives. There is also activity against the sexual forms (gametocytes) and therefore it is having some potential to reduce transmission rates.[1,2] Few methods have been reported for the estimation of these drugs as single drug or in combination with other drugs. Piperaquine Phosphate (PQP) has been reported to be estimated by HPLC[3,4], LC-MS with impurities[5], and LC-UV for stability studies. While for DHA estimation was carried out with HPLC[7-10], core enhanced Accucore column[11], LC-tandem Mass[12,13], spectrophotometry [14]. So far no analytical method was available for simultaneous estimation of PQP and DHA.

MATERIALS AND METHOD

Materials

HPLC grade Methanol (Fischer scientific), Ammonium acetate and HPLC grade Acetic acid were used. Deionized HPLC grade water was used to prepare mobile phase and diluents solutions. Both the drugs; PQP and DHA were obtained from Shreya Life Sciences Pvt. Ltd., Aurangabad, M.S., India.

Equipments

HPLC analysis was performed on a Dionex HPLC system with P680 Pump, automated sample injector, a GRACESMART RP-18 column (250 mm X 4.6 mm i.d., with particle size 5 µm) and a programmable variable wavelength UV-visible detector. Data were collected and processed using "Chromeleon version 6.0" software.

Preparation of Ammonium Acetate Buffer pH 4.6

Accurately weighed 0.3854 g of Ammonium acetate was dissolved in deionized HPLC grade water to make 10 mM buffer strength. Then pH of buffer adjusted to 4.6 using HPLC grade Acetic acid. [15]

Preparation of Mobile Phase

The two components of mobile phase; Methanol (HPLC grade) and Buffer (prepared previously) were separately filtered through a 0.45µm nylon membrane filter. They were mixed respectively in the ratio of 85:15 % v/v and sonicated for 15 min. [15]

Preparation of Standard Laboratory Mixture Solution

Accurately weighed quantity of 10 mg of PQP and 10 mg of DHA were transferred to 100 ml volumetric flask, dissolved in mobile phase and volume was made up to mark with same solvent. From stock solution suitable aliquot was transferred to 10 ml volumetric flask and diluted to mark with the mobile phase, to obtain the concentration of 10 µg/ml of PQP and 10 µg/ml of DHA. A volume of 10 µl of solution was injected. All measurements were repeated three times for each concentration.

RESULT AND DISCUSSION

Under the stated chromatographic conditions, the retention time of drugs was 2.02 min for PQP and 2.46 min for DHA. A model Chromatogram for pure laboratory mixture is shown in figure 1.

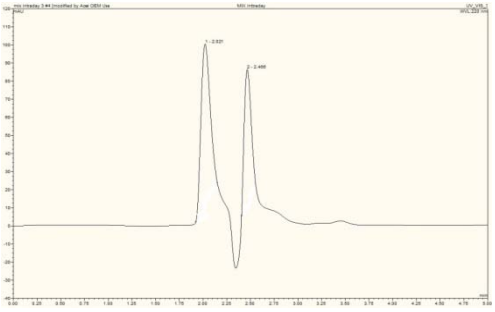

Fig. 1: Chromatogram for Pure Drug Mixture

Method Validation: [16-22]

The proposed method was validated as per ICH parameters and guidelines.

Linearity

Appropriate aliquots of the standard stock solutions of PQP and DHA were pipette out and transferred to a series of 10 ml volumetric flasks respectively.

The volume was made up to the mark with mobile phase to obtain working standard solutions of each drug separately of concentrations 5, 10, 15, 20, and 25µg/ml. From these solutions, 10 µl injections of each concentration of the drug were injected into the HPLC system three times separately and chromatogram was obtained under the conditions as finalized by developed method. The peak areas were recorded. The standard calibration curves for PQP and DHA were plotted separately as peak area Vs the respective concentration.

Table 1: Linearity study of DHA at 220 nm for HPLC

S. No.	Concentration (µg/ml)	Peak Area Mean ± S.D.[n=3]	%RSD
1	5	3.39±0.0832	2.450
2	10	6.25±0.0757	1.211
3	15	9.403±0.0404	0.429
4	20	12.38±0.0808	0.652
5	25	16.17±0.195	0.195
Average % RSD			**0.536**

Table 2: Linearity study of PQP at 220 nm for HPLC

S. No.	Concentration (µg/ml)	Peak Area Mean ± S.D.[n=3]	%RSD
1	5	4.586 ±0.0611	1.333
2	10	9.26±0.0754	0.814
3	15	14.103±0.0665	0.471
4	20	20.416±0.104	0.509
5	25	26.066±0.0709	0.261
Average % RSD			**0.677**

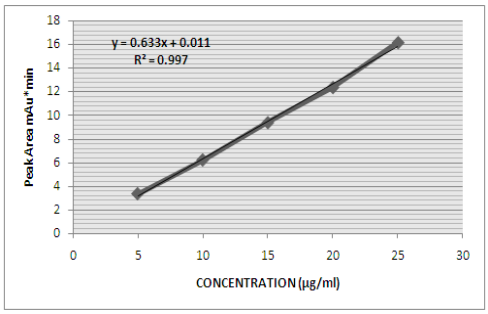

Fig. 2: Calibration Curve of Standard DHA

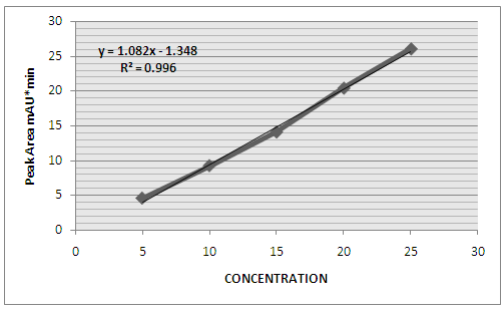

Fig. 3: Calibration Curve of Standard PQP

Accuracy

Accuracy of proposed method has been carried out by recovery studies. Recovery studies were carried out by applying the method to drug content analysis present in sample to which known amount of standard PQP and standard DHA was added at 80 %, 100 % and 120 % levels.

The technique involves addition of standard drug solution to preanalysed sample solution. The resulting sample solutions were injected onto HPLC system and chromatogram was recorded.

The results are shown in Table 3.

Precision

It is expressed as ± S.D. of series of measurements. Precision was carried out by two methods according to ICH guidelines:

1) Repeatability studies

2) Intermediate precision – variation in days and analyst.

Repeatability

It is measured by multiple injections of a homogenous sample of 10 µg/ml of PQP and 10 µg/ml of DHA that indicates the performance of the HPLC instrument under chromatographic conditions. Results are shown in Table 4.

Table 3: Results of recovery studies of PQP and DHA for HPLC

Pre-analysed Sample Solution (µg/ml)	% Level of Addition	Excess drug Added (µg/ml) (n=3)	Amount Recovered (µg/ml)	Average %Recovery	Average % RSD
PQP (10)	80	8	17.96	99.81	0.139
	100	10	19.96	99.80	0.051
	120	12	22.04	100.18	0.090
DHA (10)	80	8	17.98	99.88	0.030
	100	10	20.03	100.15	0.037
	120	12	22.00	100	0.036

Table 4: Results of Repeatability Studies of PQP and DHA for HPLC

Analyte	Amount taken (µg/ml)	Amount Found (µg/ml)±S.D. [n=6]	%RSD
PQP	10	9.93±0.0544	0.547
DHA	10	10.023±0.0169	0.168

Table 5: Results of Intra-Day and Inter-Day Precision of PQP and DHA for HPLC

Analyte Conc. (µg/ml)		Intra-Day Amount Found (µg/ml)		Inter-Day Amount Found (µg/ml)	
		Mean±S.D. [n=3]	%RSD	Mean±S.D. [n=3]	%RSD
PQP	5	4.93±0.014	0.248	4.97±0.0251	0.505
	10	10.01±0.035	0.349	9.96±0.0585	0.587
	15	14.81±0.025	0.169	14.84±0.036	0.242
		Avg.%RSD	**0.255**	**Avg. %RSD**	**0.444**
DHA	5	4.97±0.0305	0.613	5.92±0.0246	0.446
	10	9.877±0.025	0.254	9.84±0.0254	0.258
	15	14.99±0.058	0.387	14.95±0.031	0.210
		Avg.%RSD	**0.418**	**Avg. %RSD**	**0.304**

Table 6: Results of precision after variation in Analyst

Analyte	Amount taken (µg/ml)	Analyst I Avg Amount Found (µg/ml)	%RSD	Analyst II Avg Amount Found (µg/ml)	%RSD
PQP	10	9.963	0.208	9.95	0.361
DHA	10	9.89	0.288	9.93	0.374

Intermediate precision

1) Variation in days: Intra–day and Inter–day Precision

In the intra-day studies, 3 replicates of 3 different concentrations (5, 10, 15 µg/ml) of PQP and (5, 10, 15 µg/ml) of DHA were analyzed in a day and percentage RSD was calculated.

For the inter day variation studies, 3 replicates of different concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. Results are shown in Table 5.

2) Variation in Analyst

Specificity and Selectivity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

The method is quite selective. There was no other interfering peak around the retention time of PQP and DHA; also the base line did not show any significant noise.

Sensitivity (LOD and LOQ)

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD was found to be 1.15µg/ml and 0.329µg/ml for PQP and DHA respectively.

LOQ was found to be 1.39µg/ml and 0.997µg/ml for PQP and DHA respectively.

Robustness

Robustness of the proposed method was assessed by making deliberate changes in flow rate, and proportion of mobile phase which was performed by injecting sample solution containing 10 µg/ml of PQP and 10 µg/ml of DHA.

And chromatographic resolution between two drugs was evaluated. No significant change was observed during the study. Results are shown in Table7.

Table 7: Results of Robustness of PQP and DHA

Parameters	PQP (10µg/ml)	%RSD	DHA (10µg/ml)	%RSD
	RT	Peak Area	RT	Peak AREA
Mobile phase Composition (v/v) (Ammo. Acetate Buffer pH 4.6:MeOH)				
20:80	2.05	9.73	2.47	4.74
15:85	2.02	9.77 1.22	2.46	4.90 1.13
10:90	2.09	9.83	2.44	4.68
Flow Rate Variation				
1.1 ml/min	2.15	9.79	2.56	4.75
1.2 ml/min	2.02	9.84 0.457	2.46	4.79 0.613
1.3 ml/min	1.94	9.88	2.35	4.73

Table 8: Summery of Validation Parameter for RP-HPLC Estimation of PQP and DHA

Parameters	PQP	DHA
Range	5-25 µg/ml	5-25 µg/ml
Linearity		
Regression equation (y=mx+C) and correlation coefficient	y = 1.133x - 1.459 R^2 = 0.995	y = 0.690x - 0.923 R^2 = 0.999
LOD	1.15	0.329
LOQ	3.49	0.997
Recovery (% RSD)	0.173	0.093
Precision (% RSD)		
Repeatability	0.168	0.547
Intra-Day (n=3)	0.418	0.255
Inter-Day (n=3)	0.304	0.444
Ruggedness (% RSD)		
• Analyst I (n=3)	0.288	0.208
• Analyst II (n=3)	0.374	0.361
Sensitivity	Sensitive	Sensitive
Robustness	Robust	Robust

CONCLUSION

The research work concluded that the Antimalerial drugs (PQP and DHA) have been simultaneously analyzed with modern tools and techniques like HPLC. It can be further concluded that the RP-HPLC method being developed is reproducible, sensitive, precise, specific, and accurate. The mobile phase is simple to prepare and the sample recovery in formulation was in good agreement with their original claim. Statistical analysis proves that the developed method can be used for routine analysis of Piperaquine phosphate and Dihydroartemisinin in bulk.

Though the method shows good results there is need to study more about the chemical and thermal stability of Dihydroartemisinin as it have created critical problems during experimental work. Still there is wide scope for further research in this topic such as method development for the pharmaceutical formulation of PQP and DHA.

CONFLICT OF INTERESTS

Declared None

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