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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PIPERAQUINE TETRAPHOSPHATE AND DIHYDROARTEMISININ IN COMBINE DOSAGE FORMS

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ABSTRACT

The proposed method is suitable for the quantitative determination of piperaquine tetraphosphate and dihydroartemisinin in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of piperaquine tetraphosphateand Dihydro artemisinin was done by RP-HPLC. The Phosphate buffer was pH 4.6 and the mobile phase was optimized which consists of MEOH: Phosphate buffer mixed in the ratio of 70:30 % v/v. A Symmetry C18 (4.6 x 150mm, 5 m, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 273 nm.

Keywords: Symmetry C18, piperaquine tetraphosphate, Dihydroartemisinin, RP-HPLC.

I. INTRODUCTION

Piperaquine tetraphosphate

Piperaquine ($C_{29}H_{44}Cl_2N_6O_{16}P_4$) is an antimalarial agent first synthesized in the 1960's and used throughout China. Its use declined in the 1980's as piperaquine resistant strains of Plasmodium falciparum appeared and artemisinin derivatives became available. It has come back into use in combination with the artemisinin derivative Artenimol as part of the combination product Eurartesim

IUPAC Name

1,3-bis[4-(7-chloroquinolin-4-yl)piperazin-1-yl]propane.

Dihydroartemisinin

Dihydroartemisinin (also known as dihydroqinghaosu, artenimol or DHA) is a drug used to treat malaria. Dihydroartemisinin is the active metabolite of all artemisinin compounds (artemisinin, artesunate, artemether, etc.) and is also available as a drug in itself. It is a semi-synthetic derivative of artemisinin and is widely used as an intermediate in the preparation of other artemisinin-derived antimalarial drugs. It is sold commercially in combination with piperaquine and has been shown to be equivalent to artemether/lumefantrine.

IUPAC Name

(3R,5aS,6R,8aS,9R,12S,12aR)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10-ol.

Literature review reveals that there's no analytical methodology reported for the analysis of Piperaquine tetraphosphate and Dihydroartemisinin by coinciding estimation by RP-HPLC. Photometer, HPLC and HPTLC area unit the reported analytical ways for compounds either separately or together with alternative dose kind. Hence, it absolutely was felt that, there's a necessity of latest analytical methodology development for the coinciding estimation of Piperaquine tetraphosphate and Dihydroartemisinin in pharmaceutical dose kind.

Present work is aimed to develop a replacement, simple, fast, rapid, accurate, economical and consistent RP-HPLC methodology for the coinciding analysis of Piperaquine tetraphosphate and Dihydroartemisinin. The developed methodology is valid in keeping with ICH pointers.

MATERIALS

HPLC grade Methanol (Fischer scientific), Potassium dihydrogen and HPLC grade Acetonitrile were used. Deionized HPLC grade water was used to prepare mobile phase and diluents solutions. Both the drugs; Piperaquine tetraphosphate & dihydroartemisinin were obtained from Shreya Life Sciences Pvt. Ltd., Aurangabad, M.S., India.The methods will be first developed, then Validated as per ICH guidelines, then the method will be applied to the formulations.

Preparation of Phosphate buffer (PH: 4.6)

Weighed 6.8 grams of KH_2PO_4 was taken into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid**.**

Preparation of mobile phase

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of Methanol (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Preparation of the individual Piperaquine tetraphosphate standard preparation

10mg of Piperaquine tetraphosphate working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of diluent is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent**.**

Preparation of the individual Dihydroartemisinin standard preparation

10mg of Dihydroartemisinin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of diluent is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 1.0 mL from the above stock solution is pipette into a 10 mL volumetric flask and was diluted upto the mark with diluent.

Preparation of Sample Solution (Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Dihydroartemisinin and Piperaquine tetraphosphate(marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluent.

METHODOLOGY

The selected and optimized mobile phase was A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of MEOH (70%)and conditions optimized were: flow rate (1.0 ml/minute), wavelength (273 nm), Run time was 7 min. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry.

RESULTS AND DISCUSSION

The developed method of analysis was validated as per the ICH for the parameters like, linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation(LOQ).

Linearity

The linearity line was found to be 25-125µg / ml for Piperaquine tetraphosphate and Dihydroartemisinin. Correlation modules have been identified as 0.999 & 0.999, the slopes are labeled as 13644 & 8192, and 24221 & 14308 for Piperaquine tetraphosphate and Dihydroartemisininand the regression equations were calculated is shown in Fig.4&5 and results were presented in Table 4.

Precision

To check the intra-day and inter-day variation of the method, standard concentration was subjected to the proposed HPLC method of analysis. The precision of the proposed method i.e. the intra and inter-day variations in the peak area of the drug solutions was calculated in terms of percent RSD. A statistical evaluation revealed that the relative standard deviation of drugs at differentconcentration levels for 6 injections was less than 2.0. The results for intra-day and inter-day precision were presented in Table 5

Accuracy

The recovery studies were carried out for the accuracy parameter. Accuracy at different concentrations (50%, 100%, and 150%) wereprepared and the % recovery was

calculated.The percentage recovery was found to be within the limit i.e. 98-102%. The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.The results were presented in Table 6

Limit of Detection and Limit of Quantification

LOD and LOQ were determined by using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations, **LOD = 3.3 × σ / s** and **LOQ=10×σ/S**., The results were presented in Table 7.

CONCLUSION

A sensitive & selective stability indicting RP-HPLC technique have been developed & validated for the analysis of Lamivudine and Nevirapine. Depending on the on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Lamivudine and Nevirapine indicated that the developed method is specific for the estimation of Lamivudine and Nevirapine.Further the proposed RP-HPLC technique has excellent sensitivity, precision and reproducibility.

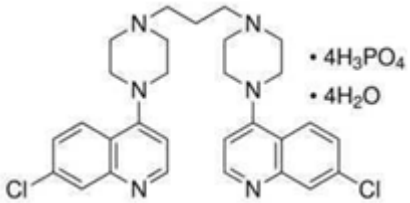

Fig. 1: Chemical Structure of Piperaquine

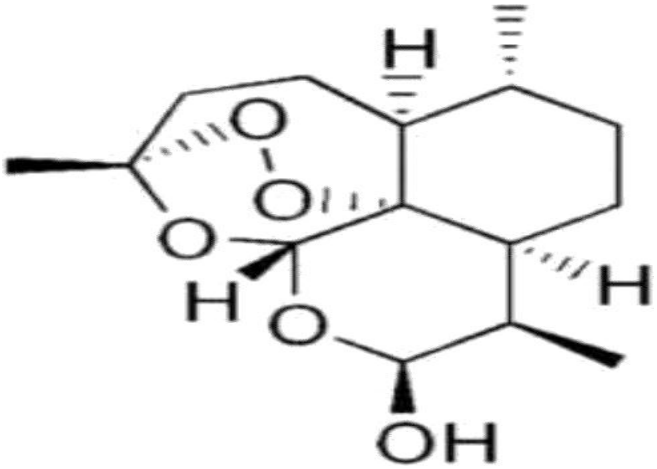

Fig. 2: Chemical Structure of Nevirapine

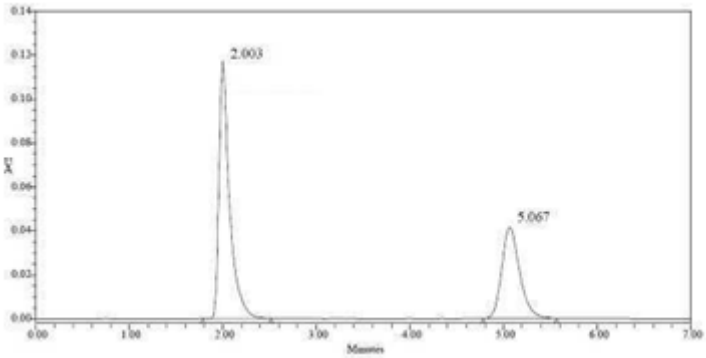

Fig. 3: Optimizedchromatogram Piperaquinetetraphosphate

and Dihyroartemisinin

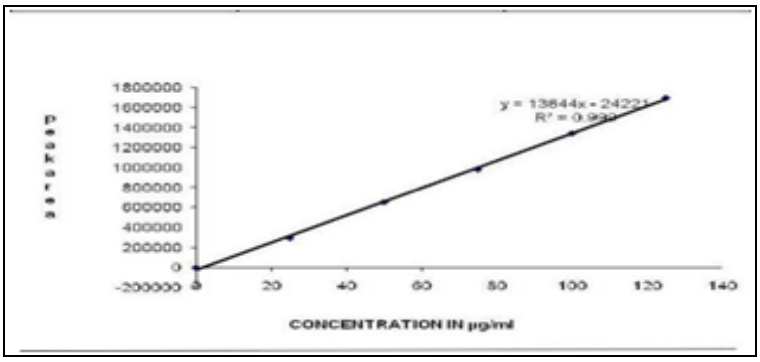

Fig. 4: Calibration curve of Piperaquine tetraphosphate

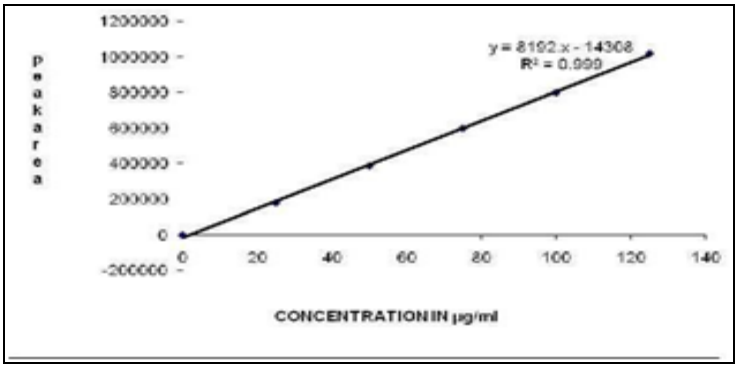

Fig. 5: Calibration curve of Dihyroartemisinin

Table 1: List of various equipments used

S.No.	Instrument	Model No.	Software	Manufacturer's
1	HPLC	Waters2695	Empower	Waters
2	UV doublebeam	UV 3000	UV Win 5	Lab India
3	Digitalweighing	BSA224SC	-	Satorius
4	pH meter	AD102U	-	Lab India
5	Ultra	SE60US	-	-
6	Suction pump	VE115N	-	-

Table 2: List of various materials used

S.No	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitril	Merck	HPLC Grade
4	Potassiumdihydrogen	Merck	A.R
5	Piperaquine tetraphosphate & dihydroartemisinin	-	-

Table 3: Optimized Chromatographic Conditions

Mobile phase	70% MeOH : 30% Phosphate Buffer pH-4.6
Wavelength	273nm
Flow rate	1.0 ml/ min.
Run time	07 min.
Column	Symmetry C.18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent

Table 4: Data of linearity

S.No	Piperaquine tetraphosphate		Dihyroartemisinin	
	Working conc. (µg/ ml)	Peak area	Working conc. (µg/ ml)	Peak area
1	25	296800	25	179891
2	50	653819	50	387781
3	75	983775	75	599708
4	100	1342535	100	799619
5	125	1694286	125	1019614
Correlation Coefficient (r)		0.999	0.999	
Slope (m)		13644	8192	
Intercept (c)		24221	14308	

Table 5: Precision data

Injection No.	Piperaquine tetraphosphate		Dihyroartemisinin	
	Retention time (min)	Peak area	Retention time (min)	Peak area
1	2.000	983375	5.116	592403
2	2.006	985049	5.124	592352
3	2.013	982956	5.143	592357
4	2.007	985219	5.136	592323
5	2.008	994145	5.138	596525
Mean		986148.8		593192
SD		4579.88		1666.25
%RSD		0.4644		0.2809

Table 6: Accuracy data

%Concentration (at specification Level)	Piperaquine tetraphosphate			Dihyroartemisinin		
	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery
50	24.9	24.8	99.8	24.9	24.6	99.8
100	49.9	49.8	99.6	49.8	49.7	99.8
150	74.8	74.8	100	74.9	74.8	99.6
Mean % Recovery			**99.8%**			**99.7%**

Table 7: Data table of LOD & LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml
Piperaquine Tetraphosphate	3.14 µg/ml	10.05 µg/ml
Dihyroartemisinin	2.75 µg/ml	9.96 µg/ml

CONCLUSION

The validated HPLC method here proved to be simple, economical, rapid, precise, accurate, and robust which can be used for the routine quality control analysis of piperaquine tetraphosphate and dihydroartemisinin in combined tablet dosage forms

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