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Analytical Method Development and Validation of Piperaquine Tetraphosphate and Dihydroartemisinin In Combine Dosage Forms

Sravanthi Bijjiga
Sree College of Pharmacy, Nayakulagudem (V), Kothagudem, Bhadradri

ABSTRACT

The estimation of Piperaquinen tetraphosphate and Dihydroartemisinin 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, 5µm, Make X Terra) column used as stationary phase. The detection was carried out using UV detector at 273 nm. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of Piperaquinen tetraphosphate and Di hydro artemisinin were found to be from 25-125 µg/ml. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Piperaquinen tetraphosphate and Di hydro artemisinin LOD and LOQ was found to be within limit. The proposed method is precise, simple and accurate to determine the amount of Piperaquinen tetraphosphate and Di hydro artemisinin in formulation. High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

Keywords: Symmetry C18, Piperaquinen Tetraphosphate, Dihydroartemisinin, RP-HPLC.

*Corresponding Author Email: sravanthi.bijjiga@gmail.com
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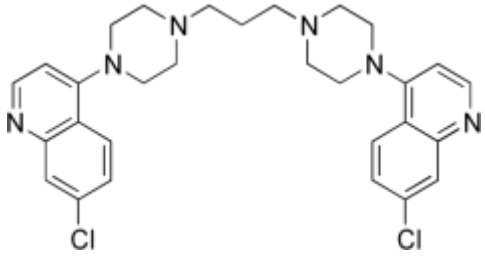
INTRODUCTION

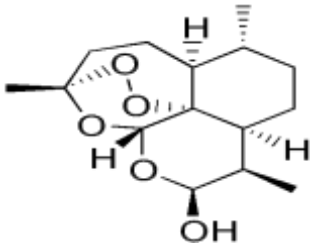
Piperaquine Tetraphosphate[3]

Piperaquine is an antimalarial drug, a bisquinoline first made in the 1960s, and used extensively in China and Indochina as prophylaxis and treatment during the next 20 years. Usage declined in the 1980s as Piperaquinen-resistant strains of P. falciparum arose and artemisinin-based antimalarial became available. However, Chinese scientists have been studying whether piperaquine can still be used therapeutically in combination with artemisinin. Piperaquine is characterized by slow absorption and a long biological half-life, making it a good partner drug with artemisinin derivatives which are fast acting but have a short biological half-life. The fixed-dose combination di hydroartemisinin-piperaquine (Eurartesim) was submitted for approval to the European Medicines Agency in 2009.



Dihydroartemisinin

Dihydroartemisinin (also known as di hydroqinghaosu, artenimol or DHA) is a drug used to treat malaria. Dihydroartemisinin is the active metabolite of all artemisinin compounds (artemisinin, articulate, 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 hate proposed mechanism of action of artemisinin involves cleavage of end peroxide bridges by iron, producing free radicals (hypervalent iron-Oxo species, epoxides, aldehydes, and carbonyl compounds) which damage biological macromolecules causing oxidative stress in the cells of the parasite. Malaria is caused by apicomplexans, primarily Plasmodium falciparum, which largely reside in red blood cells and itself contains iron-rich hemi-groups (in the form of hemozoin). In 2015 artemisinin was shown to bind to a large number targets suggesting that it acts in a promiscuous manner. Recent mechanism research discovered that artemisinin targets a broad spectrum of proteins in the human cancer cell proteome through hemi-activated radical alkylation s been shown to be equivalent to artemether/lumefartrine.



MATERIALS AND METHOD

Instrumentation:

Table1: List of Instruments

S.No.	Instrument	Model No.	Software	Manufacturer's name
1	HPLC Alliance PDA Detector	Waters2695 Waters996	Empower	Waters
2	UV double beam spectrophotometer	UV3000	UVWin5	Lab India
3	Digital weighing balance	BSA224SCW	-	Sartorius
4	pH meter	AD102U	-	Lab India
5	Ultrasonicator	SE60US	-	-
6	Suction pump	VE115N	-	-

Table 2: List of Chemicals

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Potassium di hydrogen orthophosphate	Merck	A.R
5	Piperaquine tetraphosphate & Dihydroartemisinin	-	-

TRIALS

Preparation of the individual Dihydroartemisinin standard preparation*:*

10mg of Dihydroartemisinin working standard was accurately weighed and transferred into a 10ml clean dry volumetricflaskandabout2mlof 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 ispipetteintoa10ml volumetric flask and was diluted upto the mark with diluent.

Chromoto grams Conditions

Mobile phase : Water: Methanol (50:50%v/v)

Column : Thermosil C18 (4.6*150mm) 5µm

Flow rate : 1.0 ml/min

Wavelength : 260 nm

Column temp : Ambient

Sample Temp : Ambient

Injection Volume : 10 µl

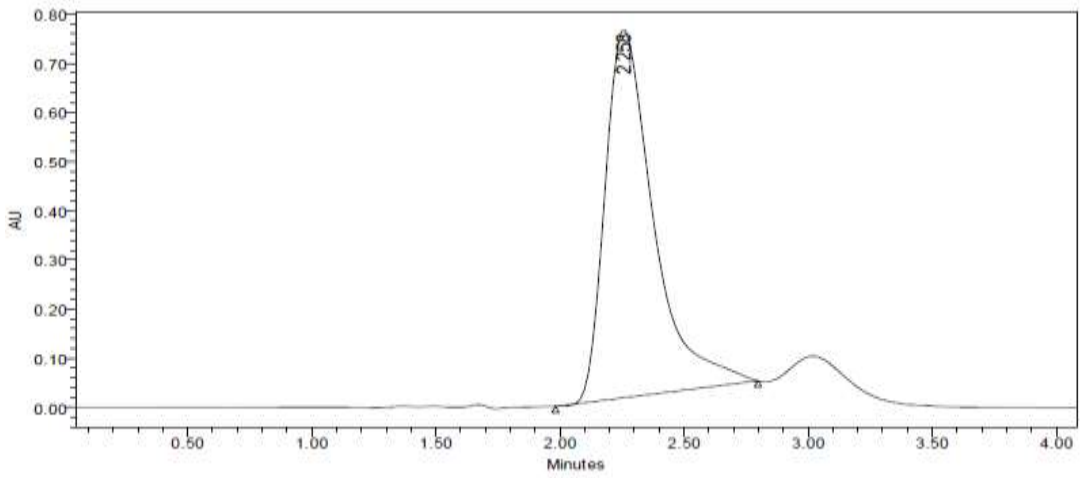


Figure: 1 Chromatogram showing trail -1

From the above chromatogram it was observed that the di hydroartemisinin peak was splitted

Trial 2:

Mobile phase : Phosphate buffer pH 4: Methanol (40:60%v/v)

Column : Termosil C18 (4.6*150mm) 5µm

Flow rate : 1.0 ml/min

Wavelength : 260 nm

Column temp : Ambient

Sample Temp : Ambient

Injection Volume: 10 µl

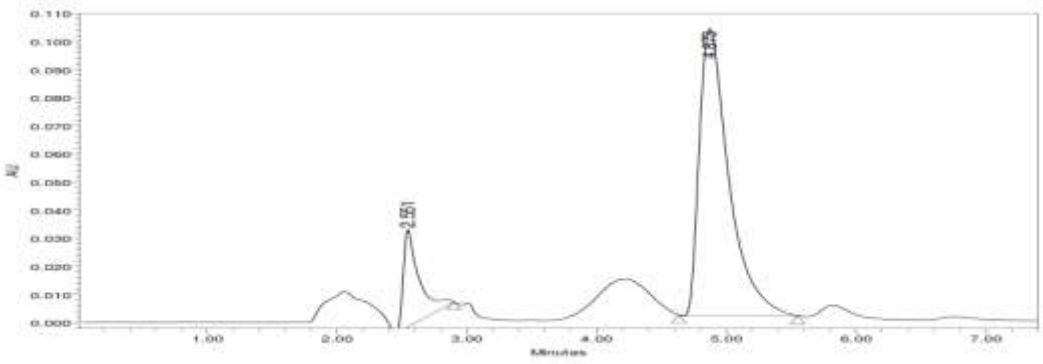


Figure: 2 Chromatogram showing trail -2

From the above chromatogram it was observed that the di hydroartemisinin and piperaquine tetraphosphate peaks are splitted

Trial 3:

Mobile phase : Phosphate buffer (0.05m) pH 4.0: Methanol (40:60%v/v)

Column : Symmetry C18 5µm (4.6*250mm) Make; waters

Flow rate : 0.8 ml/min

Wavelength : 260 nm

Column temp : Ambient

Sample Temp : Ambient

Injection Volume : 10 µl

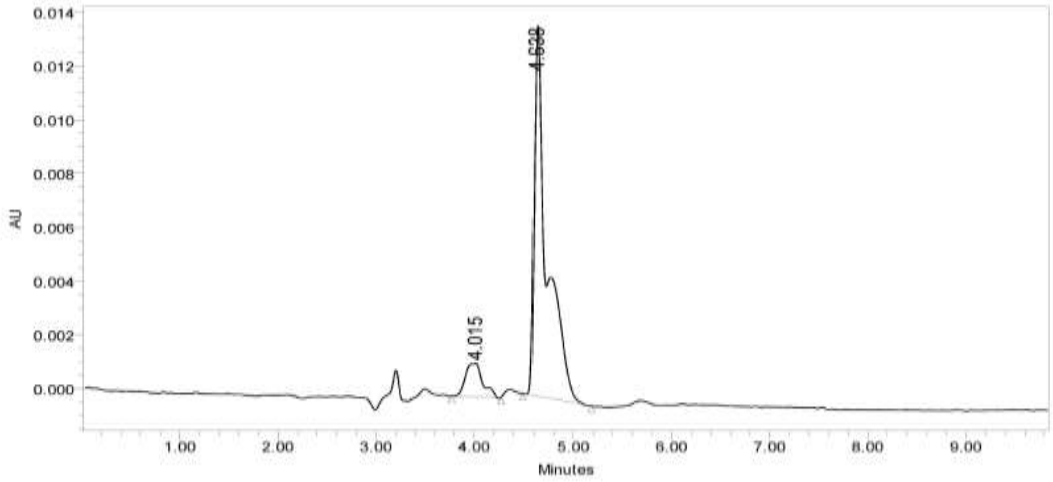


Figure: 3 Chromatogram showing trail -3

From the above chromatogram it was observed that the di hydroartemisinin and piperaquine tetraphosphate peaks are splitted

Optimized chromatogram is obtained by following conditions

Column : Symmetry C18 (4.6 x 150mm, 5µm, Make: X Terra) or equivalent

Buffer pH : 4.6

Mobile phase : 70% me oh: 30% phosphate buffer ph-4.6

Flow rate : 1 ml per min

Wavelength : 273 nm

Temperature : ambient.

Run time : 7min.

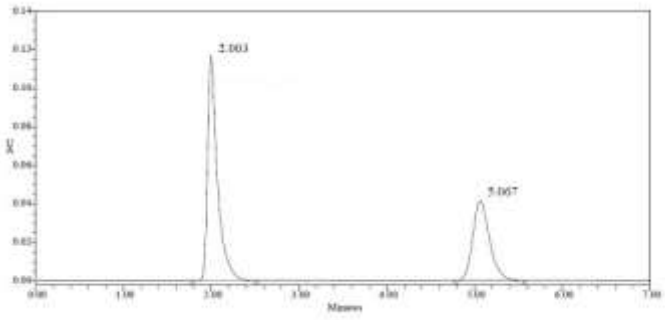


Figure: 4 Chromatogram for Piperaquine tetraphosphate and Di hydroartemisinin

From the above chromatogram it was observed that the Piperaquine tetraphosphate and Di hydroartemisinin peaks are well separated

Retention time of Piperaquine tetraphosphate – 2.003 min

Retention time of Dihydroartemisinin - 5.067 min.

Preparations and procedures:

Preparation of Phosphate buffer :(PH: 4.6):

Weighed 6.8 grams of KH2PO4 was taken into a1000ml beaker, dissolved and diluted to 1000 ml with HPLC water, adjusted the pH to4.6with ortho phosphoric acid.

Preparation of mobile phase:

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

Diluant Preparation*:*

Mobile phase is used as Diluents**.**

Preparation of the individual Piper aquine tetra phosphate standard preparation:

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

Preparation of the individual Dihydroartemisinin in standard preparation*:*

10 mg of Dihydroartemisinin working standard was accurately weighed and transferred into a10 ml clean dry volumetric flask and about 2 ml 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 diluant.

Preparation of Sample Solution :*(* Tablet)

Accurately 10 Tablets are weighed and crushed in mortar and pestle and weight equivalent to10 mg of Dihydroartem is in in and Piperaquine tetraphosphate (marketed formulation)sample into a10 mL clean dry volumetric flask and about 7 mL 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 a10 ml volumetric flask and diluted up to the mark with diluant.

Procedure*:*

10 µ Lof the standard, sample are injected into the chromatographic system and the areas for Dihydroartemisinin and Piperaquine tetraphosphate peaks are me assured and the %Assay are calculated by using the formulae.

System Suitability*:*

Tailing factor for the peaks due to Dihydroartemisinin and Piperaquine tetraphosphate in Standard solution should not be more than2.0.

Theoretical plates for the Dihydroartemisinin and Piperaquine tetraphosphate peaks in Standard solution should not be less than 2000

METHOD VALIDATION SUMMARY

Accuracy:

Preparation of standard solution (Piperaquine tetraphosphate and Dihydroartemisinin):

Accurately weighed 10 mg of Dihydroartemisininand 10mg of Piperaquine tetraphosphate working standard were transferred in to a10mLand100mlofcleandryvolumetric flasks. About7mL and 70ml of Diluents reader and sonicated to dissolve it completely and made volume upto the mark with the same solvent.(Stocksolution)Further 3 ml and 0.3 ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted upto the mark with diluents

Preparation of Sample solutions*:*

For preparation of 50%solution (With respect to target Assay concentration):

Accurately5mgofDihydroartemisinin and5mgofPiperaquine tetraphosphate working standard were weighed and transferred into a10 mL and 100ml of clean dry volumetric flask and about7mL of Diluents was added and sonicated to dissolve it completely and made volume upto the mark with the same solvent.(Stock Solution).Further 3ml and0.3ml of the above Dihydroartemisinin

And Piperaquine tetra phosphate stock solution were pipetted into a10ml volumetric flask and

diluted upto the mark with diluent

For preparation of 100%solution (With respect to target Assay concentration):

Accurately10mg of Dihydroartemisinin and10mg of Piperaquine tetra phosphate working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume upto the mark with the same solvent.(Stock Solution).

Here 3 ml and 0.3ml of the above Dihydroartemisinin and Piperaquine tetra phosphate stock solution were pipetted into a10ml volumetric flask and diluted upto the mark with diluant.

For preparation of 150%solution (With respect to target Assay concentration):

Accurately15mg of Dihydroartemisinin and 15mg of Piperaquine tetraphosphate working standard were weighed and transferred into a10mL and 100 ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume upto the mark with the same solvent.(Stock Solution).Further 3 ml and 0.3 ml of the above Dihydroartemisinin and Piperaquine tetraphosphate stock solution were pipetted into a 10 ml volumetric flask and diluted upto the mark with diluant.

Procedure*:*

The standard solution, Accuracy -50%, Accuracy-100% and Accuracy -150% solutions were injected. The Amount found and Amount added for Dihydroartemisinin &Piperaquine tetraphosphate and the individual recovery and mean recovery values were calculated.

Acceptance criteria

Core relation coefficient should be not less than 0.999.

Table 3: Results of Accuracy

Sample concentration	Sample set no	Sample area		Assay		% Recovery	
		ARTE	PIPE	ARTE	PIPE	ARTE	PIPE
50%	1	460064	276931	24.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Average Recovery					99.7%	99.7%
100%	1	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Average recovery					99.6%	99.8%
150%	1	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Average recovery					99.7%	99.8%

Acceptance criteria:

The percentage recovery at each level should be between (97-103%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence the method is accurate.

PRECISION

A) Repeatability*:*

Preparation of standard stock solution*:*

Accurately10 mg of Dihydroartemisinin and 10 mg of Piperaquine tetra phosphate working standard were weighed and transferred into a10 mL and100 ml of clean dry volumetric flasks and about 7 mL and 70 ml of Diluant was added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution)Further it was pipette (3ml and0.3ml) into 10mlvolumetricflaskanddiluteduptothemarkwith diluents.

Procedure*:*

The standard solution was injected for five times and the areas for all five injections in HPLC were measured. The %RSD for the area of five replicate injections was found to be within the specified limits. The Chroma to grams is showninFigs.7.7–7.11andresultsaretabulatedinTables7.2-7.3

Table 4: Results of method precision for Piperaquine tetraphosphate:

S. No	Sample area	Standard area	Percentage purity
1	983375	971536	101.04
2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994145	981422	101.09
Average			**100.84**
%RSD			**0.304**

Table 5: Results of method precision for Dihydroartemisinin

S. No	Sample area	Standard area	Percentage purity
1	592403	577531	101.36
2	592352	580381	101.85
3	592357	577723	102.32
4	592323	582190	101.44
5	596525	583378	101.09
Average			**101.24**
%RSD			**0.46**

Acceptance criteria:

%RSD for sample should be NMT 2 The %RSD for the standard solution is below 2, which is within the limits hence the method is precise.

B) Inter mediate Precision (Ruggedness):

To evaluate the inter mediate precision (also known as ruggedness) of the Method, precision was performed on different days by using different make column of same dimensions.

Preparation of standard stock solution*:*

Accurately10 mg of Dihydroartemisinin and10 mg of Piperaquine tetraphosphate working standard were weighed and transferred into a 10mL and100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further this Stock was pipette (3mland0.3ml) into a10ml volumetric flask and dilutes upto the mark with diluents.

Procedure

The standard solution was injected for five times and the area for all five injections measured in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits .The chromate grams are shown in Fig 7.12-7.16 and results are tabulated inTable.7.4-7.5

Acceptance criteria

The %RSD for the area of five sample injections results should not be more than2%.

Table6: Results of Intermediate precision for Piperaquine tetraphosphate

S. No	Sample area	Standard area	Percentage purity
1	979556	984395	99.30
2	982467	984039	99.64
3	979717	983976	99.36
4	978909	984278	99.28
5	981432	973915	100.57
Average			**99.63**
%RSD			**0.54**

Table 7: Results of Intermediate precision for Dihydroartemisinin

S. No	Sample area	Standard area	Percentage purity
1	583416	593403	99.12
2	583657	594352	99.01
3	584731	593357	99.52
4	583594	592673	99.61
5	597649	593671	99.12
Average			**99.27**
%RSD			**0.27**

Acceptance criteria:

%RSD of five different sample solutions should not be more than 2 The %RSD obtained is within the limit, hence the method is rugged.

Specificity

The system suitability for specific it was carried out to determine whether there is any interference of any impurities inretention time of analytical peak. The specificity was performed by injecting blank.The Chroma to grams are shown in Figure.

LOD:

LOD'scan is calculated based on the standard deviation no of the response (SD) and the slope of the calibration curve(S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined base donate standard deviation of y-intercepts of regression lines.

Formula:

$$LOD = 3.3 \text{ X } \frac{\sigma}{S}$$

Where

σ-Standard deviation(SD) S–Slope

LOQ:

LOQ' scan be calculated based on the standard deviation of the response(SD)and the slope of the calibration curve(S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of intercepts of regression lines.

Formula:

LOQ= 10 σ/Slope

Where

σ-Standard deviation

S–Slope

Linearity

Preparation of stock solution*:*

Accurately10 Tablets were weighed & crushed in mortar and pest land weight equivalentto10 mg of Dihydroartemisinin and Piperaquine tetraphosphate (marketed formulation)sample were transferred in to a 10mL clean dry volumetric flask and about 7mL of Diluant was added and sonicated to dissolve it completely and made volume upto the mark with the same solvent.(Stocksolution)

Preparation of Level–I (20 ppm of Dihydroartemisinin & 10 ppm of Piperaquine tetraphosphate):

1 ml of stock solution has taken in 10 ml of volumetric flask and diluted upto the mark with

diluent.

Preparation of Level–II (40ppm of Dihydroartemisinin & 20ppm of Piperaquine tetraphosphate):

2 ml of stock solution has taken in 10 ml of volumetric flask and diluted upto the mark with diluent.

Preparation of Level–III (60ppm of Dihydroartemisinin & 30 ppm of Piperaquine tetraphosphate):

3 ml of stock solution has taken in 10 ml of volumetric flask and diluted upto the mark with diluent.

Preparation of Level–IV (80ppm of Dihydroartemisinin & 40 ppm of Piperaquine tetraphosphate):

4 ml of stock solution has taken in 10 ml of volumetric flask and diluted upto the mark with diluent.

Preparation of Level–V (100ppm of Dihydroartemisinin & 50 ppm of Piperaquine tetraphosphate)

5 ml of stock solution has taken in10 ml of volumetric flask and diluted upto the mark with diluent.

Procedure*:*

Each level was injected into the chromate graphic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated. The chromate grams are shown in Fig.7.26-7.30and results are tabulated inTable.8,

Calibration graph for Piperaquine tetraphosphate and Dihydroartemisinin are shown inFig.7, 8.

Table 8.: Area of different concentration of Piperaquine tetraphosphate and Di hydroartemisinin

Concentration(µg/ml)	Peak area of Piperaquine tetraphosphate	Peak area of Dihydroartemisinin
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614

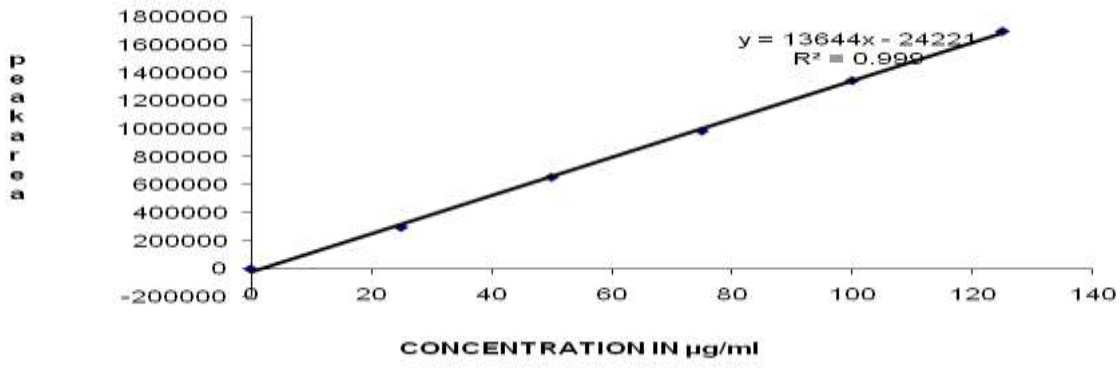


Figure 5:.Calibration graph for Piperaquine tetraphosphate at 273 nm

Figure 6:. Calibration graph for Dihyroartemisinin at 273 nm

Table 9:.Analytical performance parameters of Piperaquine tetraphosphate and Dihydroartemisinin

Parameters	Piperaquine tetraphosphate	Dihydroartemisinin
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999 The correlation coefficient obtained was 0.999 which is in the acceptance limit. The linearity was established in the range of 25 to 150µg/ml.

Range:

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and ac curtain the range of 1µg-5µg and100µg-500µg of Piperaquine tetraphosphate and Dihydroartemisinin respectively.

Robustness:

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the imp action the method.

a) The flow rate was varied at 0.8ml/mint 1.2ml/min. Standard solution 3ppm of Piperaquine tetraphosphate and 300ppm of Dihydroartemisinin was prepared and analyzed using the varied flow rates along with method flow rate. The chromate grams are shown in Fig.7.37, 7.38 and results are tabulated inTable.7.11

b) The organic composition in the mobile phase was varied from 65% to75 % standard solution 3 µg/ml of Piperaquine tetraphosphate and 300 µg/ml of Di hydroartemisinin in were prepared and analyzed using the varied mobile phase composition along with the actual mobile phase composition in the method. The chromate grams are shown in Fig.7.39, 7.40 and results are tabulated inTable.7.12

System suitability:

5 mg of Piperaquine tetraphosphate and 500 mg of Di hydro artemisinin in working standard was accurately weighed and transferred into a100ml clean dry volumetric flask and add about 20ml of diluent and sonicated to dissolve it completely and make volume upto the mark with the same solvent (Stock solution).Further10ml of Piperaquine tetraphosphate and Di hydro artemisinin was pipetted out from the above stock solution into a100ml volumetric flask and was diluted upto the mark with diluent.

RESULTS AND DISCUSSION

Optimized chromatogram is obtained by following conditions

Column	:	Symmetry C18 (4.6 x 150mm, 5µm, Make: X Terra) or equivalent
Buffer pH	:	4.6
Mobile phase	:	70% Meow: 30% phosphate buffer ph-4.6
Flow rate	:	1 ml per min
Wavelength	:	273 nm
Temperature	:	ambient.
Run time	:	7min.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Specificity can be performed in any of the following ways: Inject sample as well as other related compounds like solvents, intermediates, degradation products.

Inject separately different components of the matrix of the sample (Tablets). Subject sample to degradation studies to produce 10-30% of degradation of analyte. Confirm peak purity with DAD, IR, NMR and MS. Change the chromatographic conditions no evidence of additional compounds.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity studies should cover the range of 0-150% of the expected level of the analyte. The data is then processed using the method of least squares regression. The resulting plot, slope, intercept and correlation coefficient provide the desired information on linearity. ICH recommends that, for the establishment of linearity, a minimum of five concentrations should normally be used

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. There are three ways to determine accuracy:

Comparison to a reference standard

Recovery of the analyte spiked into blank matrix

Standard addition of the analyte

Accuracy is calculated as the percentage of recovery by the assay of known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, together with the confidence intervals.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

a. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

b. Intermediate precision

Intermediate precision expresses within laboratories variations: different day's different analysts, different equipment, etc.

c. Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies usually applied to standardization of methodology).

ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range of the procedure (i.e., three replicates of three concentrations) or using a minimum of six determinations at 100% of the test concentration.

Detection Limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

1. Based on Visual Evaluation.
2. Based on Signal-to-Noise.
3. Based on Standard Deviation of the Response and the Slope.

LOD can be expressed as:

$$\mathbf{LOD = 3.3\sigma/S}$$

Where, σ = Standard deviation of intercepts of calibration curves

S= Mean of slopes of the calibration curves

The slope S may be calculated from the calibration curve of the analyte.

Quantitation Limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Several approaches for determining the Quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

1. Based on Visual Evaluation
2. Based on Signal-to-Noise
3. Based on Standard Deviation of the Response and the Slope

LOQ can be expressed as:

$$\mathbf{LOQ = 10\sigma/S}$$

Where,

σ = Standard deviation of intercepts of calibration curves.

S= Mean of slopes of the calibration curves.

The slope S may be calculated from the calibration curve of the analyte.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been

demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range of the analytical procedure is validated by verifying that the analytical procedure provides acceptable precision, accuracy and linearity when applied to the samples containing analytes at the extremes of the range as well as within the range.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. A good practice is to vary important parameters in the method systematically and measure their effect on separation. The variable method parameters may involve temperature ($\pm 5^0$C), buffer p^H (±0.5), ionic strength of buffers, level of additives to MP, flow rate (±0.2ml/min), wavelength (±2nm).

Ruggedness:

The precision obtained when the assay is performed by multiple analysis, using multiple instruments, on multiple days, in one laboratory, different sources of reagents and multiple lots of columns should also be included in this study.

CONCLUSION

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Piperaquinen tetraphosphate and Dihydroartemisinin in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Piperaquinen tetraphosphate and 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, 5µm, Make X Terra) column used as stationary phase. The detection was carried out using UV detector at 273 nm. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of Pipe equines tetraphosphate and Dihydro artemisinin were found to be from 25-125 µg/ml. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Piperaquinen tetraphosphate and Dihydro artemisinin LOD and LOQ was found to be within limit.

The proposed method is precise, simple and accurate to determine the amount of Piperaquinen tetraphosphate and Dihydro artemisinin in formulation. High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

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