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A Validated Stability-Indicating Hplc Assay Method For Piperaquine Phosphate In Bulk Drug

Ms. Suvarna K. Lawale
Assistant Professor, Dept.of Chemistry. CSMSS Chh. Shahu College of Engineering, Chh. Sambhajinagar Maharashtra, India

Dr.Milind B.Ubale
Principal, A.E.V.P.M. Women's College, Chh.Chhatrapati Sambhajinagar Maharashtra, India. 431001
Email: suvarnalawale@gmail.com, drmilindubale@gmail.com

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ABSTRACT:
A new simple, reliable, inexpensive, and accurate reversed phase-high performance liquid-chromatographic (HPLC) assay method was developed and validated for the simultaneous quantitative determination of Piparaquine Phosphate in bulk drug. Piparaquine Phosphate is an orally active bisquinoline antimalarial drug and shows good activity against chloroquine resistant plasmodium strains. Method developed with column Waters Xterra, RP18, with dimension 250 x 4.6mm 5µm. The mobile phase containing 2.84 gm of di-sodium hydrogen phosphate was dissolved in water and 1ml of triethyl amine was added. The pH was Adjusted 7.0 with orthophosphoric acid. The buffer: acitonitrile ratio used was 30:70. The flow rate was 1.0 mL/min, column temperature was 25°C and effluents were monitored at wavelength 320 nm. The retention time of Piperaquine Phosphate was 5.6 min. Correlation co-efficient for Piperaquine Phosphate was found to be 0.99. The novel stability indicating developed method was validated as per the ICH guidelines using various parameters, for example, accuracy, precision, limit of quantification, limit of detection, robustness, ruggedness, solution stability and recovery. Relative standard deviation associated with all the parameters was less than 2%, showing compliance with the acceptance criteria of ICH guidelines.
Key Words: HPLC, Piparaquine Phosphate, Validation, Chromatographic techniques.

1. INTRODUCTION

Piperaquine Phosphate is an antimalarial drug and shows good activity against chloroquine resistant plasmodium strains, a bisquinoline first made in the 1960s, and used extensively in China and Indochina as prophylaxis and treatment during the next 20 years[1]. Piperaquine Phosphate is chemically 1,3-bis-[4-(7-chioroquinolyl-4)-piperazinyl-1]-propane phosphate [2,3].

Molecular formula of Piperaquine Phosphate is C29H35Cl2N6O4P structural formula depicted in figure 1 and molecular weight is 633.5 g/mol.

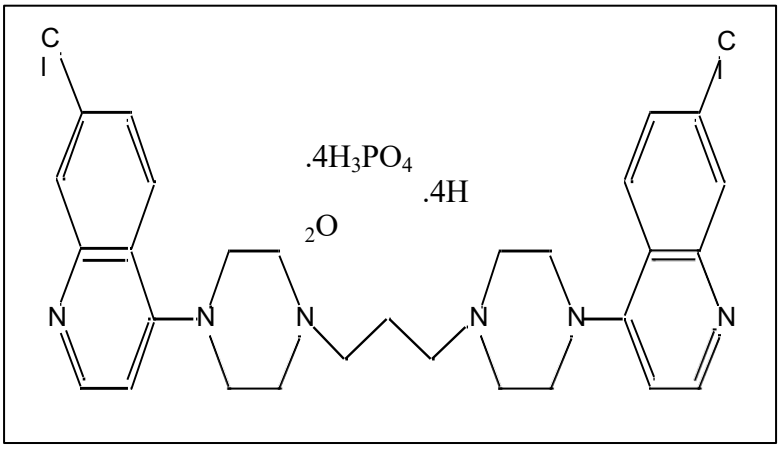

Figure1.Chemical Structure of Piperaquine Phosphate.

To analyze any drug most desirable method must be easy and sensitive with cost-effective. Aim of present work is to analyze Piperaquine Phosphate as bulk or as tablet dosage which is outcome of our continuously efforts for establishing reliable method of drugs by HPLC technique [4], according to the ICH guidelines [5-7].

2. LITERATURE SURVEY

Literature survey revealed several methods have been reported for determination of Piperaquine Phosphate in bulk as well as pharmaceutical preparations [10-15].

3. MATERIALS AND METHODS

For the separation of analyte on HPLC system Waters Xterra, RP18, 250 x 4.6mm 5µm or equivalent column was used. The instrument was equipped with a pump (2695), injector, PDA Detector (2996) and column oven. Empower software was used for data gaining. Degassing of the mobile phase was done by using an ultrasonic bath sonicator whenever necessary. For weighing the materials a Mettler Toledo (XS 205 dual range) electronic balance was used. Class 'A' Borosil glassware were employed for volumetric and general purpose in the study. The reference sample of Piperaquine Phosphate was obtained from Lupin Pvt. Ltd. Aurangabad. The tablets of Piperaquine Phosphate were obtained from the local market. Di-sodium hydrogen phosphate(AR grade, Merck),Triethyl amine(AR grade, Merck),Orthophosphoric acid(AR grade, Merck), Acetonitrile (HPLC grade, Merck), water (Milli-Q / HPLC grade) were used. Chromatographic conditions were used are shown in table 1.

4. CHROMATOGRAPHIC CONDITIONS AND PREPARATION OF SOLUTIONS:

Preparation of Acetate buffer

2.84 gm of di-sodium hydrogen phosphate was accurately weighed and transferred in 1000ml volumetric flask, dissolved in water and 1ml of triethyl amine was added ,diluted up to the mark with water. Adjusted the pH 7.0 with orthophosphoric acid.

Preparation of mobile phase:

Buffer	:	Acetonitrile (separate lines)
30	:	70

Preparation of Piperaquine Phosphate working standard solution

Weighed accurately 50.52mg of Piperaquine Phosphate working standard and transferred into a 100ml volumetric flask, dissolved and diluted up to the mark with diluents.

Preparation of Piperaquine Phosphate sample solution

Weighed accurately 50mg of Piperaquine Phosphate sample and transferred into a 100ml volumetric flask, dissolved and diluted up to the mark with diluents.

Preparation of standard solution

Weighed accurately 50.58 mg of standard and transferred it into a 100 ml volumetric flask, dissolved and diluted the volume up to the mark with diluents. Injected six replicate injections were of Piperaquine Phosphate working standard solution.

Preparation of sample solution

Weighed accurately 50 mg of test sample and transferred it into a 100 ml volumetric flask, dissolved and diluted the volume up to the mark with diluents. Prepared six samples solutions separately in similar manner.

Calculated the Assay of each sample of Piperaquine Phosphate by comparing against the working standard.

Calculated % RSD of assay values.

$$\text{Assay} = \frac{At}{As} \times \frac{\text{Ws}}{Wt} \times \frac{\text{P}}{(100 - LOD)} \times 100$$

Where,

At = Area of principal peak in sample solution,

As = Average area of principal peak in standard solution, Ws = Weight of working standard in mg,

Wt = Weight of sample in mg,

P = % Potency of working standard on as is basis.

Table 1. Optimized Chromatographic conditions.

1.	Instrument	Agilent HPLC Series 1200 with UV

		detector and auto sampler
2.	Column	Waters Xtera, RP18, 250 x 4.6mm 5µm or equivalent.
3.	Mobile phase	Buffer: Acetonitrile (30 :70)
4.	Flow rate	1.0ml/ min.
5.	Wavelength	320 nm
6.	Injection volume	20µl
7.	Column temperature	25°C
8.	Injector temperature	5°C
9.	Diluent	Water: Acetonitrile(60:40)
10.	Run Time	20 minutes

5. METHOD VALIDATION

The method was validated in acquiescence with ICH guidelines. Specificity, precision, accuracy, robustness, linearity, Limit of Quantification and Limit of Detection, system suitability and stability these parameters were determined for validation of analytical solution.

6. SPECIFICITY

The method specificity was assessed by comparing the chromatograms obtained from a saline solution containing a mixture of most commonly used excipients without the drug and another solution containing the excipients with the drug. These solutions were prepared in the mention diluents. The mixtures were filtered before injection. The saline solution and the sample solution (blank and the drug) were injected into HPLC system and the relevant chromatograms observed.

7. METHOD PRECISION

System precision:

The system precision of the method was determined by injecting six replicates of standard solution of Piperaquine Phosphate into HPLC system.

Method precision:

The precision of the procedure was determined by repeatability. In this six sample preparations were made from a single batch of Piperaquine Phosphate tablets and analyzed as per the proposed method.

Intermediate precision (Ruggedness):

Ruggedness of the method was performed by analyzing six sample preparations of same batch used under method precision as per proposed method by different analysts using different instrument and on different day. The amount of Piperaquine Phosphate in Piperaquine Phosphate tablets was determined and % RSD for % assay of was calculated, for six preparations.

Accuracy:

The accuracy was verified with known amounts of Piperaquine Phosphate (API) at about 50%, 100% and 150% of test concentration prepared in triplicate at each level. Amount of Piperaquine Phosphate was quantified and % recovery was calculated from amount found and actual amount added. % Recovery at each level was calculated.

Linearity:

Linearity of method was performed using the standard solution in a range of 50ppm to 150ppm [50% - 150% of the test concentration].

Stability in analytical solution:

Stability of Piperaquine Phosphate in analytical solution was determined by analyzing sample solution initially and also at different time intervals up to 24 hrs when the sample was stored at room temperature.

Robustness:

To evaluate robustness of the method following variations were made and the samples were analyzed in triplicate. Change in Flow rate by (10%), change in Organic content variation in mobile phase (±2mM). System suitability was evaluated in each condition and results were compared with method precision results.

Limit of Detection and Limit of Quantification: Limit of detection (LOD) is the lowest concentration of analyte that gives a measurable response. LOD is determined based on signal to noise ratio (S/N) of three times typically for HPLC methods. The limit of quantification (LOQ) is the lowest concentration that can be quantified reliably with a specified level of accuracy and precision.

8. RESULTS AND DISCUSSIONS:

Mixture of Buffer:acetonitrile (30 :70) at a flow rate 1ml/minute were found as suitable solvent system.

Table 2. Observation table for Method Precision data (sample).

Sample No.	*Wt. of sample (mg)*	*Retention time*	*Area*	*Assay (%) (as such basis)*	*Assay (%) (on dried basis)*
1	49.80	25.53	2736.54	92.69	99.31
2	50.25	25.54	2738.11	92.74	99.37
3	50.02	25.56	2740.27	92.77	99.40
4	49.98	25.54	2739.36	92.84	99.47
5	49.85	25.53	2741.08	92.83	99.46
6	49.85	25.54	2737.84	92.58	99.20
AVG	--	25.54		--	99.37

%RSD	--				0.10

Result:
% RSD of assay values is 0.10 %.

Acceptance criteria
% RSD of assay values should not be more than 1.0 %.

ACCURACY
Accuracy of Piperaquine Phosphate was studied by sample solutions prepared at three different levels as given below.

Preparation of Piperaquine Phosphate standard
Weighed 49.95 mg of Piperaquine Phosphate standard and transferred into a 100 ml volumetric flask. Dissolved and diluted up to the mark with diluents.

Table 3. Observation: Accuracy data - Standard

Working Standard	***Wt. of Standard (mg)***	***Retention Time***	***Area***
Injection-1	49..95 mg	25.53	2746.54
Injection-2	--	25.54	2748.11
Injection-3	--	25.56	2742.27
Injection-4	--	25.54	2741.36
Injection-5	--	25.53	2739.08
Injection-6	--	25.54	2737.84
AVG	--	25.54	2742.53
%RSD	--	0.13	0.05

Table 3.1 Accuracy Level-1

Accuracy Level	***Wt. of sample (mg)***	***Retention Time***	***Area***	***Assay (as such basis)***	***Assay (on dried basis)***
Level-1	(W1) 40.02	25.59	2664.14	92.62	99.24
	(W2) 40.80	25.59	2696.12	92.71	99.34
	(W3) 39.72	25.59	2659.43	93.04	99.69
	AVG	25.59	--	--	99.42

	%RSD	0.0	--	--	0.24

Table 3.2 Accuracy Level-2

Accuracy Level	***Wt. of sample (mg)***	***Retention Time***	***Area***	***Assay (as such basis)***	***Assay (on dried basis)***
Level-2	(W1) 49.82	25.59	3057.32	93.10	99.75
	(W2) 49.95	25.59	3053.81	92.69	99.31
	(W3) 50.56	25.59	3088.36	93.19	99.85
	AVG	25.59	--	--	99.64
	%RSD	0.0	--	--	0.29

Table 3.3 Accuracy Level-3

Accuracy Level	Wt. of sample (mg)	Retention Time	Area	Assay ***(as such basis)***	Assay (on dried basis)
Level-3	(W1) 60.18	25.59	3563.73	93.08	99.73
	(W2) 60.35	25.59	3521.60	93.54	100.23
	(W3) 60.75	25.59	3585.07	93.04	99.69
	AVG	25.59	--	--	99.08
	%RSD	0.0	--	--	0.30

Result:

% RSD of peak area of Piperaquine Phosphate working standard is 0.10 %. Accuracy of each sample is between 98.0 to 102.0%.

Acceptance criteria:

% RSD of peak area of Piperaquine Phosphate should not be more than 2.0 % . Accuracy of assay should be between 98.5-100.5 %.

Linearity:

Linearity of Piperaquine Phosphate was studied by injecting solutions prepared at five different levels from working standard stock solution as given below.

Preparation of Piperaquine Phosphate working standard stock solution (500 ppm): Weighed accurately 50.02 mg of Piperaquine Phosphate working standard and transferred to 100ml volumetric flask. Dissolved and diluted up to the mark with diluents, mixed well (500 ppm)

Linearity Level-1:
Transferred 5 ml of Piperaquine Phosphate working standard stock solution (500 ppm) into 50 ml volumetric flask and diluted up to the mark with diluents (50 ppm).
Linearity Level-2:
Transferred 8 ml of Piperaquine Phosphate working standard stock solution (500 ppm) into 50 ml volumetric flask and diluted up to the mark with diluents (80 ppm).
Linearity Level-3:
Transferred 10 ml of Piperaquine Phosphate working standard stock solution (500 ppm) into 50 ml volumetric flask and diluted up to the mark with diluents (100 ppm).
Linearity Level-4:
Transferred 12 ml of Piperaquine Phosphate working standard stock solution (500 ppm) into 50 ml volumetric flask and diluted up to the mark with diluents (120 ppm).
Linearity Level-5:
Transferred 15 ml of Piperaquine Phosphate working standard stock solution (500 ppm) into 50 ml volumetric flask and diluted up to the mark with diluents (150 ppm).
Injected these five levels in three replicates. Calculated % RSD and average peak area of these levels.

Table 4. Observation: Linearity data.

Injection No.	***Level-1 (50 ppm)***	***Level-2 (80 ppm)***	***Level-3 (100 ppm)***	***Level-4 (120 ppm)***	***Level-5 (150 ppm)***
1	984.66	1594.08	1995.24	2409.46	2977.56
2	985.87	1596.14	1994.65	2410.08	2977.49
3	985.87	1596.57	1996.05	2406.56	2972.57
AVG	985.46	1595.60	1995.31	2408.70	2975.87
%RSD	0.06	0.08	0.04	0.08	0.10
--	--	--	--	Squared Correlation Coefficient(r^2)	0.9997

Result:
Squared Correlation of Coefficient (r2) of PQP is 0.9997
Acceptance criteria:
Squared Correlation of Coefficient (r2) for PQP should not be less than 0.9900
Limit of Detection and Quantification

The limit of detection and the limit of quantification was estimated by injecting serial dilutions of less than1.0 ppm of Piperaquine Phosphate six replicates and calculated % RSD of Piperaquine Phosphate.

Observations: LOD/LOQ: Piperaquine Phosphate

Sample	***Level – 1***	***Level – 2***	***Level – 3***	***Level – 4***	***Level – 5***
ppm	0.5 ppm	0.25 ppm	0.10 ppm	0.05 ppm	0.02 ppm
	Area	Area	Area	Area	Area
Injection - 1	7.54	3.71	0.82	0.45	Not detected
Injection - 2	7.74	3.70	0.81	0.59	Not detected
Injection - 3	7.73	3.70	0.71	0.55	Not detected
Injection - 4	7.69	3.75	0.74	0.42	Not detected
Injection - 5	7.67	3.69	0.65	0.46	Not detected
Injection - 6	7.70	3.73	0.82	0.61	Not detected
Average	7.68	3.71	0.76	0.51	--
%RSD	0.91	0.54	9.21	15.69	--

Results:

Limit of detection for Piperaquine Phosphate is 0.05 ppm and limit of quantification is 0.10 ppm.

Acceptance Criteria:

LOD would be the lowest concentration of analyte which can be detected but when % RSD of six replicate injections should be more than 10%.LOQ would be the lowest concentration of analyte which can be quantified but when % RSD of six replicate injections should be less than 10%.

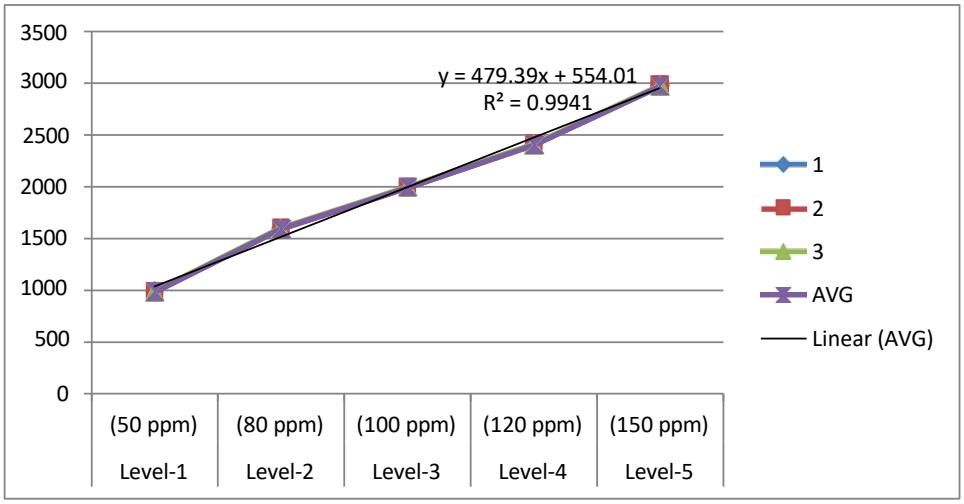

Fig.2.Linearity graph of Piperaquine Phosphate.

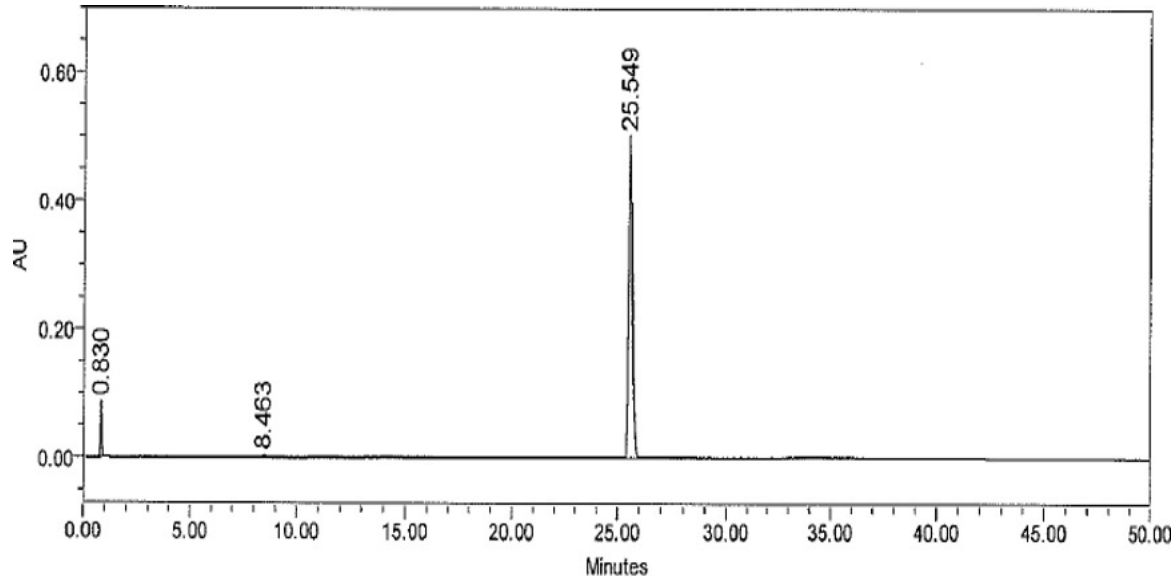

Fig.3 Chromatogram of Piperaquine Phosphate Sample

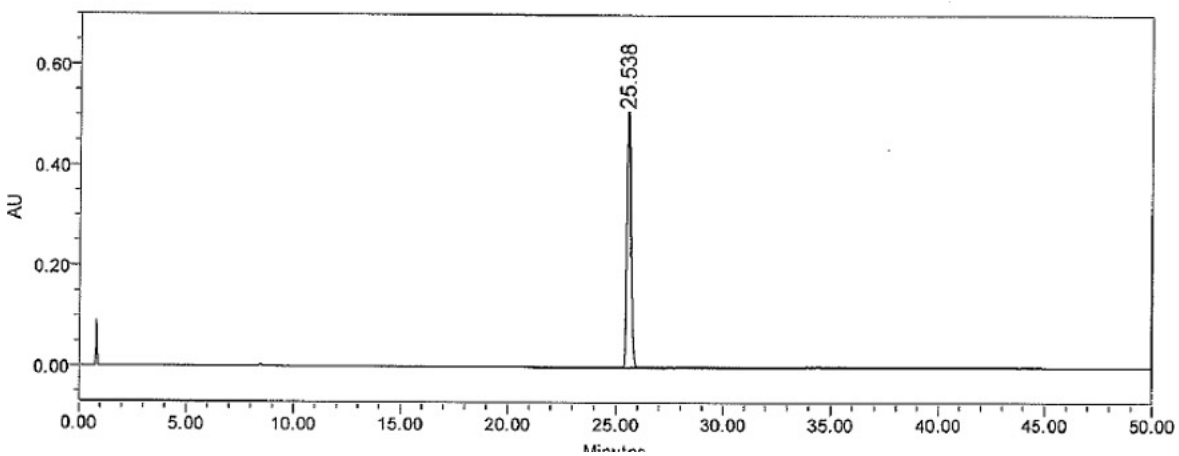

Fig.4 Chromatogram of Piperaquine Phosphate Standard

9. CONCLUSION

The method developed for quantitative determination of Piperaquine Phosphate is rapid, precise, accurate, economic and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method can be conveniently used for the assay determination of Piperaquine Phosphate in bulk drugs and pharmaceutical dosage form.

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