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Research Article

# HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ARTEROLANE MALEATE AND PIPERAQUINE PHOSPHATE IN BULK AND TABLET DOSAGE FORM

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**ABSTRACT**

A new reversed-phase HPLC method was developed and subsequently validated for simultaneous estimation of arterolane maleate and piperaquine phosphate in pharmaceutical dosage forms. Chromatography is carried out at 30°C ± 0.5°C on Inertsil Silica C18 column (250 x 4.6 mm, 5µ) with a mobile phase composed of buffer and acetonitrile (25:75) at a flow rate of 1.0 mL/min. Detection was carried out using a PDA detector at 223nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the International Conference on Harmonization guidelines. The retention times for Arterolane maleate and piperaquine phosphate are 3.1 min. and 7.2 min., respectively. The linearity range for Arterolane maleate is 30-225µg/mL and piperaquine phosphate is 150-1125µg/mL. The mean percentage recoveries of Arterolane maleate and piperaquine phosphate are 99.64% and 99.14% respectively. The relative standard deviations for three replicate measurements in three concentrations of samples in tablets are always less than 2%.

**Keywords**: RP-HPLC, arterolane maleate, piperaquine phosphate, Simultaneous estimation.

## INTRODUCTION

Malaria is a major public health problem infecting 300-500 million people worldwide and causing more than one million deaths annually globally. An endemic protozoal disease in India with an estimated 70-100 million cases each year, *Plasmodium falciparum* is responsible for half of these cases. Conditions leading to malaria treatment failure contribute to development of resistance and in clued incorrect dosing, non-compliance with duration of dosing regimen, poor drug quality, drug interactions, poor or erratic absorption and misdiagnosis. Additionally, factors that decrease the effectiveness of immune system in clearing parasite residuum after treatment also increase survivorship and intensification of resistance. Fixed dose combination of arterolane maleate (150 mg) and piperaquine phosphate (750 mg) has been recently developed and approved by FDA as a once-a day therapy for three days for the treatment of acute, uncomplicated *Plasmodium falciparum* malaria. Arterolane maleate [Figure 1] is a synthetic trioxolane compound[1]. The chemical name of arterolane maleate is cis-adamantane-2-spiro-3'-8'-[[[(2'-amino-2' methylpropyl) amino] carbonyl] methyl] 1',2',4'-trioxaspiro [4.5] decane hydrogen maleate. The molecular formula is $C_{26}H_{40}N_2O_8$ and molecular weight is 508.61. Piperaquine phosphate [Figure 2] is a synthetic bisquinoline compound. The chemical name of piperaquine phosphate is 1,3-bis[4-(7-chloroquinolyl-4)-piperazinyl-1]propane tetraphosphate tetrahydrate[2,3]. The molecular

formula is $C_{29}H_{32}C_{l2}N_6$ •$4H_3PO_4$ •$4H_2O$ and molecular weight is 999.56.

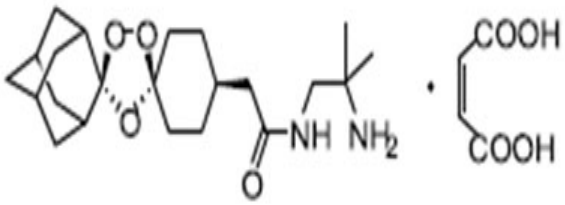


**Fig. 1: Structure of arterolane maleate**

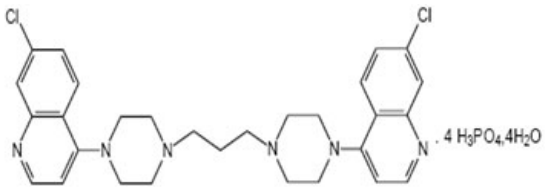


**Fig. 2: Structure of piperaquine phosphate**

## EXPERIMENTAL

### Instrumentation

Chromatography was performed with Water's 2695 HPLC system provided with Hamilton Syringe, auto sampler and 2996 Photodiode array detector. All HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Sample acquisition, analysis, and reporting were performed by Empower 2 (Waters) chromatography software.

### Reagents and chemicals

Pharmaceutically pure sample of Arterolane maleate and piperaquine phosphate were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. Acetonitrile and Methanol of HPLC grade was obtained from Merck chemical division, Mumbai and Synriam (150mg of Arterolane maleate and 750mg Piperaquine phosphate) commercial tablets were procured from the local drug market.

### Chromatographic condition

The mobile phase consisted of buffer: acetonitrile were taken in gradient at a flow rate of 1.0 ml/min. Inertsil Silica C18 column (250 x 4.6 mm, 5µ) was used as the stationary phase. Although the Arterolane maleate and piperaquine phosphate have different λ max, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 223 nm was selected as the detection wavelength for PDA detector.

### Preparation of standard stock solution

Accurately Weighed 150mg of Arterolane maleate and 750mg of Piperaquine phosphate working Standards and transferred into a 50 ml clean dry volumetric flask, add 30ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents.

### Preparation of Working Standard Solutions

Aliquot of 0.1ml, 0.25ml, 0.35ml, 0.5ml, 0.6ml and 0.75ml were pipetted out from stock into 10 ml volumetric flask separately and volume was made up to 10ml with diluent. This gives the solutions of 30µg/ml, 75µg/ml, 105µg/ml, 150µg/ml, 180µg/ml and 225µg/ml for Arterolane maleate and 150µg/ml, 375µg/ml, 525µg/ml, 750µg/ml, 900µg/ml and 1125µg/ml for piperaquine phosphate, respectively.

### Sample preparation

Twenty tablets of Synriam were weighed and crushed into fine powder. Calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 60mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 0.2 ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

### Method validation

### System suitability tests

To ensure the validity of the analytical procedure, a system suitability test was established. Data from six injections of 10µL of the working standard solutions of ART and PPQ were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time.

### Linearity

By appropriate aliquots of the standard ART and PPQ solutions with the mobile phase, six working solutions ranging between 30-225 µg/mL for ART and 150-1125µg/mL for PPQ were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of ART and PPQ to obtain the calibration curve.

### Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of ART and PPQ

to which known amounts of standard ART and PPQ corresponding to 50%, 100% and 150% of label claim were added. The accuracy expressed as the percentage of analyte recovered by the proposed method.

**Precision**

Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of ART and PPQ. Determinations were performed on the same day as well as well as on consequent days.

**Limit of detection and the limit of quantification**

Limit of detection (LOD) and limit of quantification (LOD) of ART and PPQ were determined by calibration curve method. Solutions of both ART and PPQ were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations.

**LOD = (3.3 ×Syx)/b and**
**LOQ= (10.0×Syx)/b**

Where Syx is residual variance due to regression; b is slope.

**Robustness**

The robustness of the method was performed by deliberately changing the chromatographic conditions. The organic strength was varied by ±5%, column temperature was varied by ±5$^0$c and the flow rate ±0.1mL.

**RESULT AND DISCUSSION**

**Method development**

Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol: Water, Acetonitrile and Water as mobile phases, in which both the drugs did not responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH: 3.0 both drugs eluted with better separation.

Thereafter, buffer: acetonitrile were taken 25:75 at a flow rate of 1.0 ml/min. Inertsil Silica C18 column (250 x 4.6 mm, 5μ) was used as the stationary phase was selected to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 210nm to 280nm. The wavelength at which both Arterolane maleate and piperaquine phosphate showed maximum absorption at 223nm was selected as the detection wavelength for PDA detector. The chromatogram obtained was shown in the figure 3.

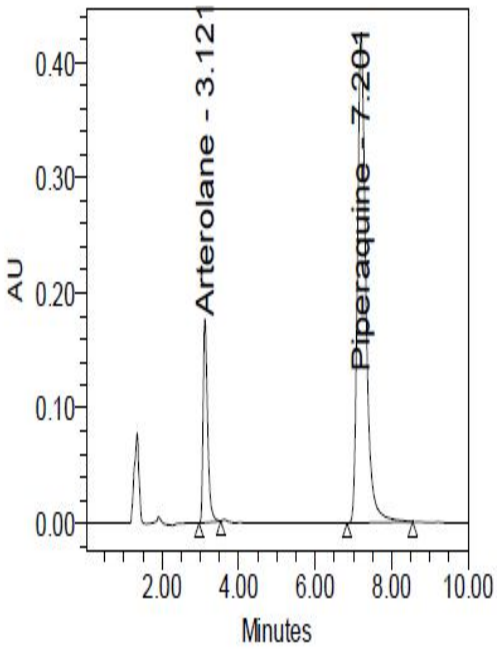


**Fig. 3: Representative chromatogram of Arterolane maleate and piperaquine phosphate**

**Method Validation**

**System suitability**

System suitability parameters such as number of theoretical plates, HETP and peak tailing were determined. The results obtained are shown in table-1.

**Table 1: System suitability of ART and PPQ**

| Parameters | ART | PPQ |
|---|---|---|
| RT | 3.1 | 7.2 |
| No of theoretical plates | 3686 | 5255 |
| Tailing Factor | 1.4 | 1.3 |
| Average Area | 1433727 | 6527425 |
| SD | 13040.0 | 20559.8 |
| %RSD | 0.9 | 0.3 |

**Linearity**

ART and PPQ showed a linearity of response between 30-225 µg/mL for ART and 150-1125µg/mL for PPQ and the represented by Figure 4A & Figure 4B.

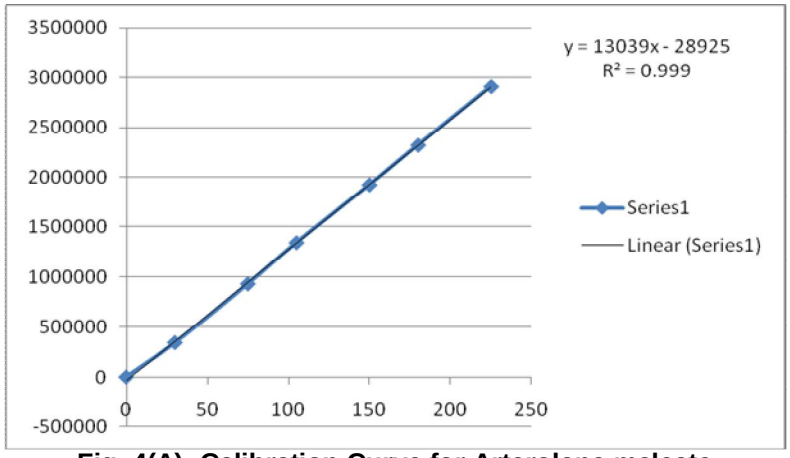


**Fig. 4(A): Calibration Curve for Arterolane maleate**

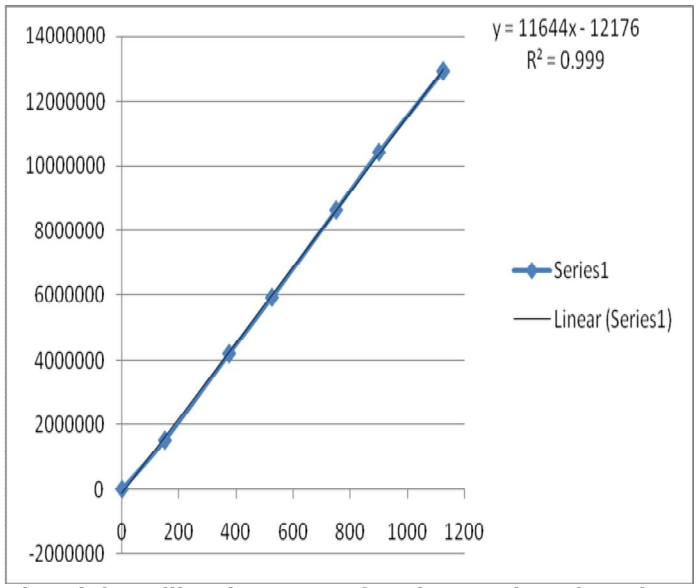


**Fig. 4(B): Calibration Curve for piperaquine phosphate**

**Accuracy**

Recovery studies were performed to validate the accuracy of developed method. To pre-analyzed sample solution, a definite concentration of standard drug was added and recovery was studied. These results are summarized in table- 2.

**Table 2: Results of Recovery Experiments of ART and PPQ**

| Spiked drug conc. (ug/ml) | | Recovery (%) | |
|---|---|---|---|
| ART | PPQ | ART | PPQ |
| 75 | 375 | 99.57 | 98.20 |
| 75 | 375 | 99.50 | 98.18 |
| 75 | 375 | 99.98 | 98.42 |
| 150 | 750 | 99.76 | 99.11 |
| 150 | 750 | 99.16 | 99.26 |
| 150 | 750 | 99.30 | 99.33 |
| 225 | 1125 | 100.22 | 100.16 |
| 225 | 1125 | 99.71 | 99.44 |
| 225 | 1125 | 99.60 | 100.14 |
| Statistical parameters | Mean | 99.64 | 99.14 |
| | SD | 0.323 | 0.749 |
| | %RSD | 0.3 | 0.8 |

**Repeatability**

Six replicates of standard concentrations were analyzed in same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in table- 3.

**Intermediate Precision**

Six replicates of standard concentrations were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits (RSD < 2) as shown in table-3.

**Table 3: Results of Precision of ART and PPQ**

| Sample No. /Statistical parameters | Repeatability (%Assay) | | Day to Day (%Assay) | |
|---|---|---|---|---|
| | ART | PPQ | ART | PPQ |
| Sample 1 | 100.40 | 99.25 | 99.5 | 102.25 |
| Sample 2 | 99.56 | 100.92 | 100.67 | 101.01 |
| Sample 3 | 99.67 | 100.40 | 99.39 | 99.83 |
| Sample 4 | 99.16 | 100.64 | 98.92 | 103.39 |
| Sample 5 | 99.02 | 99.86 | 100.44 | 102.03 |
| Sample 6 | 100.49 | 99.50 | 101.04 | 99.83 |
| Mean | 99.71 | 100.096 | 99.99 | 101.39 |
| SD | 0.6161 | 0.665 | 0.838 | 1.425 |
| %RSD | 0.6 | 0.7 | 0.8 | 1.4 |

**Robustness**

As per ICH norms, small, but deliberate variations, by altering the Flow rate, column temperature and concentration of the mobile phase were made to check the method's capacity to remain unaffected. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature.

**Stability of sample solution**

The sample solution injected after 24 hr did not show any appreciable change.

**LOD and LOQ**

LOD and LOQ for ART were 0.247 and 0.750 µg/mL respectively and for PPQ were 0.134 and 0.408 µg/mL, respectively.

**Tablet Analysis**

Content of ART and PPQ found in the tablets by the proposed method are shown in Table 4.

**Table 4: Results of HPLC Analysis of Tablets**

| Drug | Label claim | Amount found | %Assay |
|---|---|---|---|
| Arterolane maleate | 150mg | 149.50 | 99.71 |
| Piperaquine phosphate | 750mg | 750.67 | 100.09 |

## CONCLUSION

RP-HPLC method was developed and validated for simultaneous estimation of arterolane maleate and piperaquine phosphate in tablet dosage form. The regression value was found to be 0.999 for both arterolane maleate and piperaquine phosphate, which shows the response, is linear from 30-225µg/mL for arterolane maleate and 150-1125µg/mL for piperaquine phosphate. Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of arterolane maleate and piperaquine phosphate. The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 10 minutes for eluting both arterolane maleate and piperaquine phosphate. So, this method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

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