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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FAROPENEM IN BULK AND PHARMACEUTICAL FORMULATION USING THE RP-HPLC METHOD

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the determination Faropenem in Bulk and pharmaceutical dosage form. The column used was Symmetry C18 (4.6 x 150mm, 5 µm, Make: ODS) or equivalent in isocratic mode, with mobile phase containing phosphate buffer adjusted the pH-4.0 with orthophosphoric acid and Acetonitrile in the ratio (70:30%v/v) the flow rate was 0.9 mL/ min and eluents was monitored at 317 nm. The retention time Faropenem was 3.051 min, respectively. The linearity for Faropenem was in the range of 40-120 µg/ml respectively. The recovery of Faropenem was found to be 100.1%, respectively. The proposed method was validated and successfully applied to the estimation of Faropenem in tablet dosage form.

Keywords: Validation, RP-HPLC, Faropenem

INTRODUCTION

Faropenem is chemically 6-(1-hydroxyethyl)-2-[(2R)-tetrahydrofuran-2-yl]-2,3-didehydropenam -3-carboxylic acid. Faropenem is a novel β-lactam antimicrobial agent sharing structural similarities with both the penicillins and cephalosporins. It exhibits a broad spectrum of activity that includes Gram-negative, Gram-positive and some anaerobic bacteria. The primary mode of action of faropenem is consistent with that of other β-lactam antibiotics, namely binding to penicillin-binding proteins[1-3]. Faropenem has been shown to demonstrate high stability to a number of β-lactamases, including TEM-1, SHV-1 to -5, TEM-3 to -9 and the β-lactamase produced by Staphylococcus aureus. Literature survey revealed that only a few analytical methods such as high performance liquid chromatography (HPLC) method have been reported[4-6]. Hence, a new sensitive and efficient HPLC method was developed and validated for the assay of the drug in tablets. The structure of Faropenem is shown in Figure 1.

MATERIALS AND METHODS

A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used Symmetry C18 (4.6 x 150mm, 5 µm, Make: ODS) or equivalent A Bandline sonerex sonicator was used for enhancing dissolution of the compounds. An Adwa digital pH meter was used for pH adjustment. Analytically pure Faropenem was obtained as gift samples from Hetro drugs Ltd., Hyderabad, India. Acetonitrile, methanol, water (E. Merck, Mumbai, India) were of HPLC grade, while ortho-phosphoric acid and potassium dihydrogen phosphate (S. D. Fine Chemicals, Mumbai, India) were of Analytical grade used for the preparation of mobile phase.

Preparation of mobile phase and stock solutions

Potassium dihydrogen phosphate was weighed (2.72 g) and dissolved in 1000 ml of water. Finally the pH was adjusted to 4.0 with ortho phosphoric acid. The solution was sonicated for 5 minutes and filtered using Whatman filter paper. For the estimation of Faropenem from the tablets, twenty tablets Average weight was calculated and their contents were mixed thoroughly. The powder equivalent to 10mg was weighed accurately and transferred into a 10ml volumetric flask, dissolved and dilute up to mark with diluent. Mix well and filter through 0.45µm filter.

Chromatographic conditions

A reverse phase C18 column equilibrated with mobile phase phosphate buffer-Acetonitrile in the ratio (70:30%v/v) pH adjusted to 4.0 with ortho phosphoric acid was used. Mobile phase flow rate was maintained at 0.9 mL/min and eluents was monitored at 317 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 5 min. Appropriate aliquot of Faropenem stock solutions was taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 40, 60, 80,100,120 µg/mL of Faropenem The solution was injected using a 20 µl fixed loop system and chromatograms were recorded. Calibration curve was constructed by plotting average peak area versus concentrations and regression equations were calculated for Faropenem.

Determination of Faropenem dosage form

For the estimation of Faropenem from the tablets, twenty tablets were taken and their contents were mixed thoroughly. Average weight was calculated. Tablet content or the powder equivalent to 10mg was weighed accurately and transferred into a 10ml volumetric flask, dissolved and dilute up to mark with diluent. Take above solution 0.8 ml in 10 ml volumetric flask dilute up to mark with diluent (80ppm). Mix well and filter through 0.45µm filter. The solution was injected at above chromatographic conditions and peak areas were measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve. The method was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness.

Accuracy

The accuracy of the method was determined by calculating recovery of Faropenem by the normal method. Known amount of Faropenem was added to a pre quantified sample

solution, and the amount of Faropenem was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Precision

The intraday and inter day precision study of Faropenem was carried out by estimating the corresponding responses 5 times on the same day and on different days. The results are reported in terms of relative standard deviation. The Repeatability studies were carried out by estimating response of 5 different concentrations of Faropenem and results are reported in terms of relative standard deviation (%RSD)

Specificity

Commonly used excipients were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Detection limit and quantitation limit

Baseline noise obtained from blank injection is 46 µV. Signal to Noise ratio for the determination of detection limit for Faropenem is 2.95 and quantitation limit is 10.3.

Robustness

Robustness of the method was studied by Change in Organic Composition in the Mobile Phase.± % 10 and the flow rate 1.0 and 0.8 ml/min instead of 0.9 ml/min.

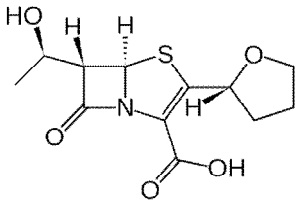

Figure 1: Structure of Faropenem

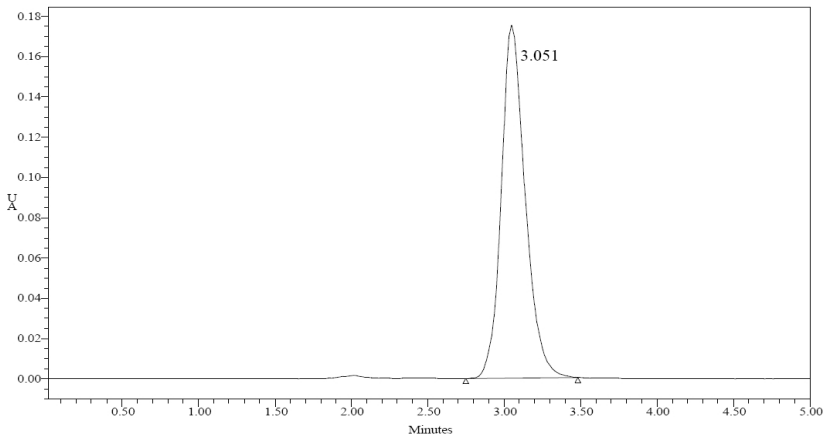

Figure 2: HPLC chromatogram of Faropenem in optimized chromatographic conditions

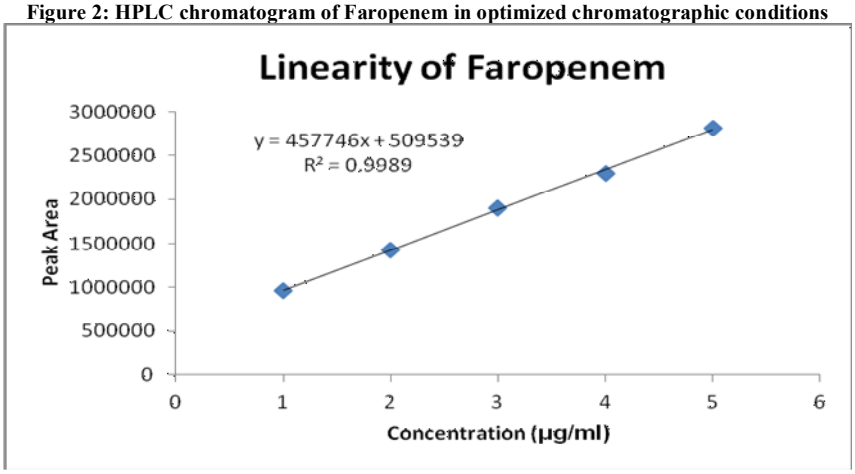

Figure 3: Linearity curve for Faropenem

Table 1: Validation parameters and data for proposed method

Validation parameter of Faropenem	Results
Linearity	40-120 µg/mL
Regression coefficient (r^2)	0.999
Limit of detection (µg/mL)	0.03
Limit of quantitation (µg/mL)	0.12
*Accuracy (% recovery)	100.1%
**Precision (%RSD)	0.06
**Intermediate precision (%RSD)	0.11
Assay value (%)	99.3
System suitability parameter	
Tailing factor	1.2
Number of theoretical plates	2883.0

* Replicates of three concentration levels (in three determinations); ** Five repetitive injections of same homogeneous sample

Table 2: Accuracy Results

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1918358	5.0	5.07	101.4%	100.1%
100%	3774514	10.0	9.98	99.8%	
150%	5630870	15.0	14.8	99.2%	

Table 3: System Suitability Results

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2060.7	1.3
2	0.9	2883.0	1.2
3	1.0	2052.3	1.2

* Results for actual flow (0.9 ml/min) have been considered from Assay standard

Table 4: Pression Results

Injection	Area
Injection-1	1913251
Injection-2	1913193
Injection-3	1910432
Injection-4	1913220
Injection-5	1912015
Average	1912422
Standard Deviation	1229.1
%RSD	0.06

Table 5: Intermediate Precision Results

Injection	Area
Injection-1	1905408
Injection-2	1907100
Injection-3	1908062
Injection-4	1909218
Injection-5	1911173
Average	1908192
Standard Deviation	2173.8
%RSD	0.11

RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on asymmetric factor and peak area obtained for Faropenem. The mobile phase phosphate buffer- Acetonitrile in the ratio (70:30%v/v) adjusted to pH 4.0 using ortho phosphoric acid was found to be satisfactory and gave symmetric peak for Faropenem the retention time for Faropenem was 3.051 min respectively (Figure 2).

The calibration curve for Faropenem was obtained by plotting the peak area of Faropenem versus the concentration of Faropenem over the range of 40-120 µg/ml, and it was found to be linear with r2 = 0.999. The detection limit for Faropenem was 0.03µg/ml respectively. The quantitation limit for Faropenem was 0.12µg/ml respectively, which suggests that a nanogram quantity of the compound can be estimated accurately. The validation parameters are summarized in (Table 1). The recovery Faropenem was found to be 100.1% respectively. The system suitability test parameters are shown in (Table 1). The liquid chromatographic method was applied to the determination of Faropenem in dosage form. The results for Faropenem were comparable with the corresponding labeled amount.

CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of Faropenem using simple mobile phase with low buffer concentration compared to the reported method. The method gives short analysis time (<5 min). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Faropenem in dosage form.

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