AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Stress Degradation studies on Faropenem Sodium and Development of a Validated method by UV. Spectrophotometer in bulk and pharmaceutical dosage form

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ABSTRACT

To develop and validate a simple, precise, accurate and stability indicating UV method for estimation of faropenem sodium. UV, HPLC and many more experiment were carried out by taking single drug and also by combining with other drug. However, such type of studies was not reported. In the developed methods for analysis and stress degradation, faropenem sodium was found to have the absorbance maxima at 300nm. Method A involved method development and validation and method B involved forced degradation study. In these methods methanol was used as a solvent. Linearity was observed in the concentration range of 10-50µg/ml. validation experiments were performed to demonstrate system suitability, specificity, precision, linearity, accuracy, robustness, LOD and LOQ as per International Conference on Harmonization guidelines. Furthermore, stability studies of faropenem sodium were carried out under acidic, alkali, oxidation and thermal degradation as per stability indicating assay methods. The result of analysis have been validated and recovery studies were carried out using a standard addition method by adding specific amount(80%,100% and 120%) and show recovery studies in range(99.34-101.24)%. The proposed method can be successfully applied for method development, validation and stability study of faropenem sodium.

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Received 10 December 2016, Accepted 19 December 2016

Please cite this article as: Varma K *et al.*, Stress Degradation studies on Faropenem Sodium and Development of a Validated method by UV. Spectrophotometer in bulk and pharmaceutical dosage form. American Journal of PharmTech Research 2017.

INTRODUCTION

Penem derivatives are a developing group of β-lactam antibiotics. The newest penem derivatives are characterized by a broad spectrum of antibacterial efficiency resulting from the trans orientation of the hydroxyethyl side chain at C6. The newest penem derivatives include: carbapenem analogs – dorypenem (EU, US) and biapenem (Japan) and a thiopenem analog; faropenem.[1]

Faropenem Sodium is 1-4 Monosodium (5R,6S)-6-[(1R)-1-hydroxyethyl]-7-oxo-3-[(2R)-tetrahydrofuran-2-yl]-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate hemipentahydrate. The antibacterial spectrum of Faropenem includes Gram-positive, Gram-negative and some anaerobic bacteria. Faropenem Sodium is a sodium salt of novel β-lactam antimicrobial ,used to treat bacterial sinusitis, pneumonia, bronchitis and skin infections. Faropenem Sodium occurs as white to light yellow crystals or crystalline powder. It is freely soluble in water and in methanol, slightly soluble in ethanol.[2-5]

As with other penems, faropenem induces bactericidal effects by binding to PBPs and inhibiting bacterial cell wall synthesis. These bactericidal effects were found to be affected by the nature of the tetrahydrofuran side chain, with an unsaturated derivative showing reduced activity compared with that of the saturated derivative (faropenem).[6]

Faropenem is also less susceptible to the actions of DHP-1 than are the carbapenems imipenem and meropenem; it has been proposed that the absence of a protonable group in the 2-side chain of faropenem, in contrast to the presence of such groups in the equivalent side chains of the carbapenems, is responsible for this phenomenon. Finally, faropenem is resistant to the effects of many bacterial β-lactamases. This property is thought to be due to the 1-(R)-hydroxyethyl group at C6 of the bicyclic molecule.[7]

Faropenem is used to treat bacterial sinusitis, pneumonia, bronchitis and skin infections. Literature survey revealed only HPLC methods[8-10] was reported for the assay of faropenem. To the best of our knowledge, there is no visible spectrophotometric method has been published so far by exploring thoroughly the analytically useful functional groups in faropenem. Hence we have made an attempt to develop and validate a simple, economic rapid and accurate method.[8-10]

MATERIALS AND METHOD

Apparatus

- Digital balance: Contech and K-Ray instrument Pvt Ltd.
- Hot air oven: Dolphin ™ max temp. 500ºc

- Sonicator
- A double beam UV- Spectrophotometer (Agilent) with Cary win UV software

Material

Marketed Formulation

Orpenem was purchased from an open market for this study which contains 200 mg of Faropenem sodium.

Reagent and chemical

Methanol (analytical reagent) was used as a solvent and was procured from S. D. Fine Chemicals Ltd.

Stock Solution

Accurately weighed 100 mg of faropenem sodium was transferred to a 100ml volumetric flask. Then, the volume was made up to the mark by methanol. The standard stock solution of faropenem sodium (1000µg/ml) was prepared.

The 10ml was taken out and transferred to another 100ml volumetric flask and the volume was made up to the mark by methanol. The II stock solution of faropenem sodium (100µg/ml) was prepared.

Absorption maxima method

For the selection of analytical wavelength, 10µg/ml solution of faropenem sodium was prepared by appropriate dilution of stock solution and scanned in the spectrum mode from 800 to 200nm. From the spectrum of drug, λ_{max} of faropenem sodium 300nm was selected for the analysis. Figure 2

Preparation of working standard solution

From the stock solution of 100µg/ml, working standard solution of drug was prepared by appropriate dilution and was scanned in the entire UV range to determine the λ_{max}.Standard solution was prepared in the concentration range of 10, 20, 30, 40, 50µg/ml.The absorbance of each solution was measured at 300nm against methanol as a blank. [Table 1]

Preparation of calibration curve

The standard calibration curve for faropenem sodium was plotted by taking concentration of drug on the X-axis and absorbance in Y-axis [Figure.1].

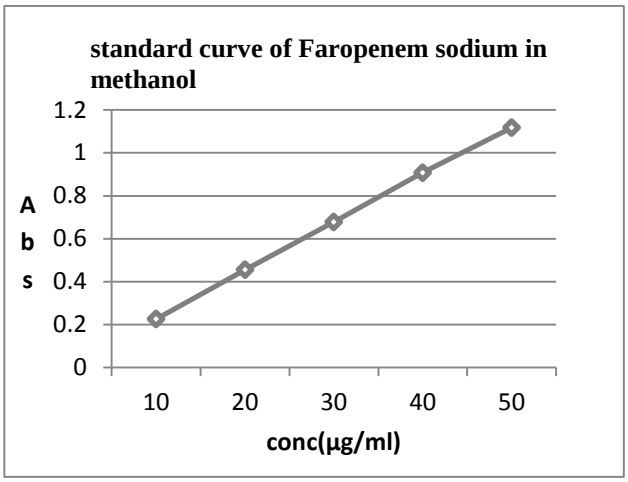

Figure 1: Standard curve of Faropenem Sodium in methanol

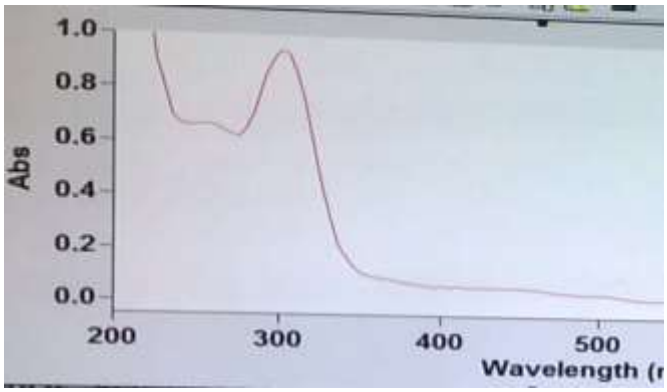

Figure 2: Spectral Scan of Faropenem sodium

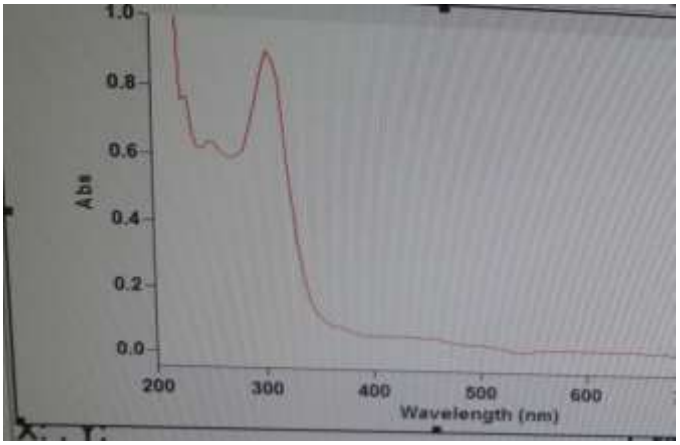

Figure 3: Spectral scan of faropenem sodium with excipients

Preparation of sample solution

The proposed method was applied to analyze commercially available Orpenem tablet. Ten tablets were weighed and powdered .The amount of powdered tablet equivalent to 10mg of faropenem sodium was weighed accurately and transferred to a 100 ml volumetric flask. Then 50 ml of methanol was added and allowed to sonicate for 15-20 min and the volume was made up to mark

with methanol. The solution was then filtered through a Whattman filter paper. This filtrate was diluted suitable with a solvent to get the solution of 10µg/ml concentration. The absorbance was measured against blank solution; the drug content in each tablet was estimated by using the standard graph.

METHOD A: METHOD VALIDATION

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of faropenem sodium. Beer- Lambert's law was found to follow in the concentration range 10-50µg/ml [Table 1 and Table 2].

Table 1: Linearity table

Concentration	λmax	Absorbance
10 µg/ml	300nm	0.226
20 µg/ml	300nm	0.455
30 µg/ml	300nm	0.678
40 µg/ml	300nm	0.908
50 µg/ml	300nm	1.117

Table 2: Optical characteristics of the linear graph

Optical characters	Values
Absorbance maxima	300nm
Beer's limit	10-50µg/ml
% RSD	0.520
Regression equation	y=0.022x-1.336
Slope	0.022
Intercept	1.336
Correlation coefficient	1/0.999

Specificity

Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. The specificity of the method was conducted to prove that the drug is free from interference of commonly used tablet excipients. The drugs were studied in the presence of various excipients e.g. lactose, talcum powder, corn starch, magnesium stearate which were prepared in the proportion corresponding to those which were used in the final dosage form [Figure 3]

Accuracy

To ascertain the accuracy of the proposed method, recovery studies were carried out by the standard addition method at three different level (80%, 100%, 120%) of bulk sample of faropenem sodium to the previously analyzed solution of formulation of the concentration of 40µg/ml. The

percentage recovery for faropenem sodium by all method was found to be in the range of (98.6-99.4) % [Table 3.]

Precision

The precision of the method was achieved by replicate (n=6) analysis of tablet preparation [Table 4]. The precision was also studied in terms of intraday change in absorbance of drug solution on the same day and inter day change on three different days over a period of 1 week. The intraday and inter day variation were calculated in terms of percentage relative standard deviation and the result are given in Table 4 (A and B).

Robustness

This procedure was carried out by changing the λ_{max} ±5. The results are given in Table 5.

METHOD B: METHOD STRESS DEGRADATION

Acid Degradation

First 0.1N hydrochloric acid was taken in 10ml volumetric flask and then accurately weighed 10mg bulk drug was dissolved in it. To solubilize the drug, few drops of methanol was added and then the volume was made with 0.1N HCl. The solution was refluxed for 4h at 70˚C in water bath. Initially at 0h 0.1ml of this solution was withdrawn and the volume was made to 10ml with methanol. The specific amount of solution samples were withdrawn at every hour and the absorbance was measured in a UV spectrophotometer. [Table 6]

Alkali Degradation

Accurately weighed 10mg bulk drug was taken in a 10ml volumetric flask and diluted with 0.1 N NaOH. Then, this solution was refluxed for 4h at 70°C in a water bath. Initially at 0hours, 0.1 ml of this solution was withdrawn and the volume was made up to10 ml with methanol. The absorbance was measured after every hour by withdrawing the required amount of the sample. Then, scanning was performed with a UV spectrophotometer [Table 7]

Thermal Degradation

A specific amount of bulk drug was taken in a cleaned petridish and dried. This was placed in the oven at 70˚C for 4 hours. At every hour ,10 mg of bulk drug was taken from the petridish and 1000 ppm solution with methanol was prepared. After then the required concentration of 10µg/ml was prepared and the absorbance was measured using UV spectrophotometer and percentage of degradation was calculated [Table 8]

Oxidative Degradation

10mg of bulk drug was weighed accurately and 2-3 drops of methanol were added to make the drug soluble. Then, the volume was made up by 3% hydrogen peroxide and placed in a cupboard

for 4h. At one hour interval specified amount of sample was taken and required concentration of 10µg/ml was prepared. It was scanned in a UV Spectrophotometer [Table 9].

RESULTS AND DISCUSSION

The main objective of this work was to develop and validate the stability indicating UV method for faropenem sodium in pharmaceutical dosage form.

The absorbance maxima of faropenem sodium were found to be at 300nm and linearity was observed in the concentration range of 10-50µg/ml. The percentage assay of Orpenem tablet by above validated method was found in the range of 98.6-99.4%. Standard deviation was found to be less than ±2 and the coefficient of variance was found to be less than ±1indicating the precision of the methods. Values of standard deviation and coefficient of variation were satisfactory low indicating the accuracy of the method.

For forced degradation studies, the absorbance in all stressed condition were found to decrease for repeated times and percentage degradation was found out. The degradation behavior was done at initial 0h and total degradation after 4h. Therefore it was found that the drug faropenem sodium undergoes degradation in all stressed condition.

Based on the results obtained it was inferred that the proposed method was accurate, precise, reproducible, economical and could be employed for routine analysis of faropenem sodium in its pharmaceutical dosage form.

Table 3: Accuracy Table

Level (%)	Formulation (µg/ml)	Added pure Drug(µg/ml)	Amount recovered (µg/ml)	%Recovery	Statistical analysis Mean	SD	% RSD
80	10	8	17.825	99.02	98.90	0.109	0.110
80	10	8	17.801	98.89			
80	10	8	17.785	98.80			
100	10	10	20.018	100.09	99.67	0.374	0.375
100	10	10	19.911	99.55			
100	10	10	19.875	99.37			
120	10	12	21.955	99.79	99.83	0.201	0.200
120	10	12	22.015	100.06			
120	10	12	21.926	9.66			

Table 4: Precision

Concentration µg/ml	Absorbance at 300nm	Calculated amount	Statistical analysis
10	0.223	9.94	Mean = 9.87
10	0.220	9.89	
10	0.220	9.89	SD=0.089
10	0.222	9.93	

10	0.221	9.91	%RSD= 0.901
10	0.224	9.7	

A. Intraday precision

Concentration µg/ml	Absorbance at 300nm	Calculated amount	Statistical analysis
10	0.219	9.86	Mean= 9.89
10	0.223	9.93	
10	0.220	9.87	SD= 0.090
10	0.222	9.92	
10	0.223	9.93	%RSD= 0.910
10	0.220	9.7	

B. Inter day precision

Concentration µg/ml	Day 1	Day2	Day3	Statistical analysis
10	0.221	0.220	0.216	Mean=9.84
10	0.223	0.217	0.219	
10	0.219	0.219	0.216	SD=0.047
10	0.221	0.218	0.217	
10	0.220	0.221	0.219	%RSD= 0.477
10	0.222	0.218	0.218	
Mean	0.221	0.218	0.219	
Amt found	9.9	9.787	9.83	

Table 5: Robustness Table

Conc. µg/ml	Absorbance at 295nm	Amount found	Statistical analysis	Concentration µg/ml	Abs at 305nm	Amt. found	Statistical analysis
10	0.205	9.06	Mean=9.075	10	0.207	9.144	Mean= 9.13
10	0.202	9.02		10	0.203	9.081	
10	0.204	9.03	SD=0.047	10	0.205	9.122	SD=0.035
10	0.207	9.11		10	0.208	9.18	
10	0.203	9.09	%RSD=0.517	10	0.209	9.16	%RSD=0.384
10	0.208	9.14		10	0.206	9.14	

Table 6: Acid Degradation Study

Name	Absorbance	Concentration	%Degradation
At 0h	0.205	10	00
At 1h	0.192	9.3	7.0
At 2h	0.153	7.14	25.9
At 3h	0.110	5.32	46.8
At 4h	0.098	4.73	52.7

Table 7: Alkali Degradation Study

Name	Absorbance (nm)	Concentration (µg/ml)	%Degradation
At 0h	0.228	10	00
At 1h	0.202	8.85	11.5
At 2h	0.185	8.10	19.0
At 3h	0.142	6.21	37.9
At 4h	0.110	4.85	51.5

Table 8: Thermal Degradation Study

Name	Absorbance	Concentration	%Degradation
At 0h	0.225	10	00
At 1h	0.185	8.22	17.8
At 2h	0.131	5.82	41.8
At 3h	0.118	5.24	47.6
At 4h	0.102	4.52	54.8

Table 9: Oxidative Degradation Study

Name	Absorbance (nm)	Concentration (µg/ml)	%Degradation
At 0h	0.219	10	00
At 1h	0.206	9.40	6.1
At 2h	0.199	9.08	9.2
At 3h	0.165	7.52	24.8
At 4h	0.138	6.28	37.2

Table 10: **Forced degradation study result for faropenem sodium after 4 hours**

Conditions	% degradation
0.1N HCl	52.7
0.1N NaOH	51.5
Thermal degradation	54.8
3% H_2O_2	37.2

CONCLUSION

The proposed method is specific in estimating the commercial formulation without interference of excipient and additives. Hence, this method can be used for routine determination of faropenem sodium in the bulk sample and pharmaceutical formulation. The proposed method for stability study shows that there was appreciable degradation of faropenem sodium under stress conditions.

REFERENCES

1. K.N. Shurek, R. Wiebe, J.A. Karlowsky, E. Rubinstein, D.J. Hoban, G.G. Zhanel, Expert Rev. Anti-infect. Ther. 5 (2007) 185.
2. Schurek K N, Wiebe R, Karlowsky J A, Rubinstein E, Hoban D J and Zhanel G G, Expert Rev Anti Infect Ther., 2007, 5(2), 185-198.
3. Fuchs P C, Barry A L and Sewell D L, Antimicrob Agents Chemother., 1995, 39(7), 1472–1479.
4. Inoue E and Mitsuhashi S, Antimicrob Agents Chemother., 1994, 38(9), 1974- 1979
5. Japanese pharmacopoeia.14th edition 2006. Pp. 472-473.
6. Ishiguro M, Iwata H, Nakatsuka T, et al. Synthesis and in vitro activity of novel 2-chiral substituted penems. J Antibiot 1988;41:1685-93.

7. Amyes SGB, Thomson CJ. Faropenem, the first furanem. [abstr]. Presented at the 1st international meeting on penems, carbapenems and related compounds, 'nems 2001. Venice, Italy, December 13-15, 2001.
8. Sun Ya-Xin, Zhao Li-Mei, Qiu Feng and He Xiao-Jing, *Chin J New Drugs Clin Remed.*, 2007-10.
9. Zhang Qiang, Jia Zheng-ping, Wang Rong, Fan Peng-Cheng and Chen Min, *China Pharmacy*, 2009-17.
10. Rizwana Iffath, Vanitha Prakash K and Krishna Mohan G, *Int Res J Pharm*, 2012, **3(12),** 81-83.