Spectrophotometric Methods for the Determination of New Oral Penem (Faropenem) in Pure and in Formulations

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Abstract: Three simple, precise, accurate and economical spectrophotometric methods in ultraviolet and visible region were developed for the determination of faropenem in pure and formulations have been described. The method A is based on the reduction of ferric ion into ferrous ion by the mentioned drug in the presence of 1,10-phenanthroline to form a highly stable orange red colored ferrion complex measured at 510 nm. The method B is based on the oxidation of MBTH with Fe(III) followed by coupling with mentioned drug to form a highly stable violet colored chromogen measured at 610 nm. The method C is based on the reduction of Folin Ciocalteau (FC) reagent in alkaline medium by faropenem leading to the formation of intense blue colored chromogen measured at 740 nm. The results of analysis for all the three methods have been validated statistically and by recovery studies. The proposed methods were simple, sensitive and economical for the quantitative determination of faropenem and were successfully employed for the estimation in pure and in formulations.

Keywords: Faropenem, Ferrion complex, MBTH and F.C.reagent

Introduction

Faropenem1-3 (5R,6S,8R,2’R)-2-(2’-tetrahydrofuryl)-6-hydroxyethylpenem-3-carboxylate, is a novel β-lactam antimicrobial with a penem (furanem) structure used to treat bacterial sinusitis, pneumonia, bronchitis and skin infections. Literature survey revealed only HPLC methods4-8 was reported for the assay of faropenem in plasma. 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. The three methods described here are based upon exploring thoroughly the analytically useful functional groups in faropenem using visible spectrophotometry.
Experimental

Shimadzu UV-Visible double beam Spectrophotometer (model 1601) was used for spectral studies. All chemicals were of analytical grade. 1,10-Phenanthroline (Merck), MBTH (sd. fine), Folin-Ciocalteau reagent (sd. fine), Fe(III)Cl$_3$ (A.R.), $\text{o}$-phosphoric acid (A.R.), sodium hydroxide (A.R.), sodium carbonate (A.R.) and hydrochloric acid (sd. fine) were used. Distilled water was used to prepare all solutions and in all experiments.

Reagents

The reagents; Fe(III)Cl$_3$, o-phenanthroline, o-phosphoric acid, MBTH (3-methyl-2-benzothiazilinone hydrazone hydrochloride), Fe(III)Cl$_3$, NaOH, HCl, Folin-Ciocalteau reagent (FC) and sodium carbonate (Na$_2$CO$_3$) were prepared using distilled water in the requisite concentrations.

Preparation of standard drug solution

Stock standard solution

Accurately weighed quantity of faropenem (100 mg) was dissolved in methanol (10 mL) in a volumetric flask (100 mL) and volume made up to the mark with distilled water.

Working standard solution

Prepared by diluting standard stock solution (10 mL for method A; 50 mL for Method B&C) with distilled water to get concentrations of 100 $\mu$g.mL$^{-1}$ for method A, 50 $\mu$g.mL$^{-1}$ for method B and method C respectively.

Procedure for the assay of faropenem in pharmaceutical dosage forms

Ten tablets of the faropenem drug were weighed and powdered and a quantity of the powder equivalent to 200 mg was transferred into a 100 mL volumetric flask, dissolved in 10 mL of methanol, stirred well for 2 minutes. The solution was mixed well by shaking for 10 minutes and then make up to the mark with distilled water. The solution was filtered. The filtrate was quantitatively diluted with distilled water to yield concentrations in the linear range of the assay of faropenem by the proposed methods.

Proposed procedures for the estimation of faropenem

Method A

Aliquots (0.5-2.5 mL, 100 $\mu$g.mL$^{-1}$) of standard faropenem were transferred into a series of 25 mL calibrated flasks and then solutions of FeCl$_3$ (1.5 mL) and o-phenanthroline (2.0 mL) was added successively. The total volume in each flask was brought to 10.0 mL with distilled water and heated for 30 minutes in a boiling water bath at 90 $^\circ$C. After cooling to room temperature, 2.0 mL of o-phosphoric acid was added, the volume in each flask was made up to the mark with distilled water. The absorbance of colored complex solution was measured after 5 minutes at 510 nm against reagent blank prepared similarly.

Method B

Aliquots (0.5-2.5 mL, 50 $\mu$g.mL$^{-1}$) of standard faropenem were transferred into a series of 10 mL calibrated tubes and then 1.0 mL of water, 0.5 mL of 0.5% MBTH and 0.5 mL of 0.1 N NaOH were added to each tube. The contents were heated for 10 minutes in a water bath at 100 $^\circ$C and cooled for 5 minutes in a water bath at 15 $^\circ$C. Then 0.5 mL of 1 N HCl and 2.0 mL of FeCl$_3$ solution were added successively and kept aside for 1 h. The absorbance was measured at 610 nm against reagent blank.
**Method C**

Delivered aliquots of standard faropenem solution (0.5-2.5 mL, 50 µg mL⁻¹) into a series of 25 mL calibrated tubes and the volume was adjusted to 3.0 mL with distilled water. To each of the test tubes, 5.0 mL of Na₂CO₃ and 1.5 mL of FC reagent were added and kept aside for 15 minutes. The volume was adjusted to the mark with distilled water. The absorbance was measured at 740 nm against a reagent blank prepared under identical conditions.

In all the above methods, a calibration curve was prepared by plotting the absorbance versus the concentration and the unknown was read from the calibration curve, or deduced using a regression equation, calculated from Beers law data.

**Results and Discussion**

In Method A, the ferric ion was reduced by phenolic OH group of the drug to ferrous ion, which reacts with 1, 10-phenanthroline and forms ferrion complex (Scheme 1) which exhibiting λ_max at 500 nm. In method B, MBTH loses two electrons and one proton on oxidation, forming the eletrophilic intermediate which has been postulated to be the active coupling species. The intermediate reacts with faropenem to form colored species (Scheme 2) exhibiting λ_max at 610 nm. In method C, the reduction of heteropoly acid, phosphomolybdotungstic acid, the well known Folin-Ciocalteau reagent (FC), by the faropenem in the presence of alkali thereby producing one or more possible reduced species which have a characteristic intense blue colored chromogen with λ_max at 740 nm.

**Step – II**

Faropenem + Fe(III) → Oxidation products of Cef + Fe(II) + Fe(III)(Unreacted)

Fe(III)+ o-Phosphoric acid → Complex (Unreactive)

**Scheme 1. Method –A**

**Scheme 2. Method - B**
The optimum conditions were established by varying one parameter at a time and keeping the other parameters fixed and observing the effect produced on the absorbance of the chromogen. For Method A & B, the reactions of colored species formation were slow at room temperature 25 °C and requires longer time for completion. Hence, efforts were made to accelerate by carrying out the reaction at higher temperatures. It was observed that the maximum color intensity was obtained by heating the reaction mixture at 90 °C on a boiling water bath for 30 minutes, for method A and at 100 °C on boiling water bath for 5 minutes, for method B. For method C, room temperature 37 °C is sufficient for the development of colored species. The absorbencies remained constant at room temperature for more than 8 h for Method A and B respectively. In Method C, the color was found to stable for more than 6 h at room temperatures.

The Beer’s law limits were obeyed over the concentration range of 5.0 - 25.0 µg.mL⁻¹ for method A, 2.5 -12.5 µg.mL⁻¹ for Method B and for Method C respectively. The proposed procedures are validated by determining various optical parameters, which are listed in Table 1. The linearity, intercepts and the slope have been calculated using regression equation $Y = a + bC$, where $Y$ represents optical density, ‘C’, the concentration of the drug in µg.mL⁻¹ and ‘a’ and ‘b’ represents intercepts and slope respectively.

**Table 1.** Optical characteristics and precision

<table>
<thead>
<tr>
<th>Optical Characteristics</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{max}$, nm</td>
<td>510</td>
<td>610</td>
<td>740</td>
</tr>
<tr>
<td>Beer’s law limits, µg.mL⁻¹, (C)</td>
<td>5-25</td>
<td>2.5-12.5</td>
<td>2.5-12.5</td>
</tr>
<tr>
<td>Molar absorptivity, L.mol⁻¹.cm⁻¹</td>
<td>8.54×10³</td>
<td>2.44×10⁴</td>
<td>5.23×10³</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/cm²)-0.001 abs units</td>
<td>0.0171</td>
<td>0.0126</td>
<td>0.0229</td>
</tr>
<tr>
<td>Regression equation($Y = a + bC$)*</td>
<td>0.0174</td>
<td>0.0350</td>
<td>0.0113</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0035</td>
<td>0.0019</td>
<td>0.0035</td>
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<tr>
<td>Intercept (a)</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.8191</td>
<td>0.8181</td>
<td>0.7555</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.8599</td>
<td>0.8589</td>
<td>0.7931</td>
</tr>
<tr>
<td>Confidence limits with 0.05 level</td>
<td>1.3485</td>
<td>1.3469</td>
<td>1.2438</td>
</tr>
<tr>
<td>Confidence limits with 0.01 level</td>
<td>1.3485</td>
<td>1.3469</td>
<td>1.2438</td>
</tr>
</tbody>
</table>

*Y is the absorbance and C is the concentration µg.mL⁻¹, **For six measurements

**Table 2.** Estimation of faropenem in pharmaceutical dosage forms

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labeled amount, mg</th>
<th>Amount obtained by the proposed method, mg</th>
<th>%Recovery of proposed methods**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method A</td>
<td>Method B</td>
<td>Method C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>199.64</td>
<td>199.89</td>
</tr>
</tbody>
</table>

*Average of six determinations, **Mean and standard deviation of six determinations

The precision and accuracy of the proposed methods were tested by carrying out the determination of six replicates of pure and dosage samples of the drug, whose concentration lie within Beer’s law range. The values of standard deviation (% R.S.D.) and percent range
of error (0.05 level and 0.01 level confidence limits) were calculated for the above three methods are presented in Table 1. Application of the proposed methods to the determination of faropenem in its formulations (Farobact - 200 mg) was successfully made and the results are presented in Table 2. The excellent recoveries obtained indicated the absence of any interference from the excipients.

**Conclusion**

The proposed methods were found to be simple, economical, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing faropenem showed no interference from the common. There it is concluded that the proposed methods involve the formation of highly stable colored species which makes it easier for the determination of faropenem in pharmaceutical dosage forms for routine analysis.

**Acknowledgement**

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**References**