Stability Indicating HPLC Determination of Doripenem in Pharmaceutical Dosage Forms

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ABSTRACT

A simple, selective, precise and stability indicating high performance liquid chromatographic (HPLC) method of analysis of Doripenem in pharmaceutical dosage form was developed and validated. The chromatographic conditions comprised of a reversed phase C18 column (250 × 4.6 mm, 5µm) with a mobile phase consisting of a mixture of acetonitrile and ammonium acetate (0.012M aqueous solution, pH:6.73) in the ratio of 15:85. Flow rate was 0.5ml/min. Detection was carried out at 295nm. The retention time of Doripenem was 7.13min. Doripenem was subjected to acid and alkal hydrolysis, oxidation, photochemical and thermal degradation. The linear regression analysis data for the calibration plot showed good linear relationship in the concentration range of 10 – 100 µg/ml. The value of correlation coefficient, slope and intercept were 0.99944, 48.36 and -25.026 respectively. The method was validated for precision, accuracy, ruggedness and robustness. The drug undergoes degradation under acidic, basic, photochemical and thermal degradation conditions. All the peaks of degraded products were resolved from the active ingredient. The assay of Doripenem in the human plasma and mouse serum are also reported by HPLC methods.

Doripenem is not official in any Pharmacopoeia and there is no monograph containing methods to characterize or quantify Doripenem. Such methods could offer official parameters to guarantee the validity of the assay. Hence, there is a need for simple, rapid and reproducible method for the routine analysis of Doripenem in pharmaceutical dosage forms.

The present study describes the development and validation of an HPLC method for assay of Doripenem. The HPLC method was studied following official guidelines, evaluating the main parameters and the procedures that can be applied to consider a stability indicating assay. The International Conference on Harmonization (ICH) requires stability-indicating methods to be used for assay of a drug in stability test samples. Stability-indicating methods should be suitable for determination of the drug during hydrolysis (at various pH values), oxidation, photolysis and thermal degradation.

According to FDA guidance document, a stability indicating method accurately measures the active ingredients without interference from degradation products, process impurities, excipients or other potential impurities.

MATERIALS AND METHODS

Chemicals and Reagents

Doripenem monohydrate Reference Standard was supplied by Akorn India Private Limited, Himachal Pradesh, India.
Acetonitrile HPLC grade was procured from Avantor Performance Materials, Philipsberg, USA.

High purity water was prepared with Elix-milli-Q system.

Ammonium acetate AR procured from Merck, Germany.

Sodium hydroxide, hydrochloric acid and hydrogen peroxide were procured from Qualigens, India.

**Instrumentation**

The HPLC instrument used was AGILENT 1260 RRLC system equipped with a photodiode array detector PDA 1290 infinity.

**Chromatographic Conditions**

Chromatographic separation was achieved on a reverse phase column Phenomenax C₁₈ (250 × 4.6 mm, 5µm) at ambient temperature using a mobile phase consisting of a mixture of 0.012M ammonium acetate solution (pH 6.73) and Acetonitrile in the ratio of (85:15) at a flow rate of 0.5ml/min. Detection was carried out at 295nm. The mobile phase system after preparation was filtered through a membrane filter (0.22µm) and sonicated for 10 minutes. The pH of the mobile phase was set at 6.73. Injection volume used for assay and degradation studies was 20µl. The retention time of Doripenem was 7.13 min.

**Standard Preparation**

Stock solution of Doripenem (1mg/ml) was prepared by dissolving appropriate amounts of the compounds in methanol. The solution was stored in the dark under refrigeration at 4°C and was found to be stable for several weeks.

A series of working standard solutions of Doripenem were prepared by the appropriate dilution of the above mentioned stock solution in the mobile phase to get concentration range of 10.0 -100.0 µg/ml. Doripenem standard solutions were found to be stable during the analysis time. (Fig 1.)

**Sample Preparation**

A quantity of Doripenem powder for injection (INAREM 500mg) equivalent to 20mg of Doripenem was dissolved in water and made up to volume in a 100ml volumetric flask. The solution was sonicated for 20 minutes. A 1ml aliquot was transferred to 10ml volumetric flask and diluted to volume with water. 20µl of the solution was injected into the HPLC system. Peak area was measured at retention time of 7.13 min. (Fig 2.)

![Figure 1: Chromatogram of Doripenem Standard](image1)

**Preparation of Calibration Graph**

Linearity of response for Doripenem assay method was determined by preparing and injecting solutions having concentrations from 10 to 100µg/ml of Doripenem.

**System Suitability**

The system suitability test was performed to ensure that the HPLC method was suitable to the analysis intended. A standard solution containing 20µg/ml of Doripenem was injected in triplicate. Chromatographic parameters including peak area, retention time, theoretical plates, and tailing factors were measured and the relative standard deviation (RSD) for each parameter was determined.

**Method Validation**

The HPLC method was validated by evaluation of the analytical parameters including specificity, linearity, precision, accuracy and robustness. The stability indicating capability was determined by forced degradation conditions, including testing heat, light, oxidation and acid and basic degradation.

**Specificity**

Specificity was examined for the non-degraded and degraded samples. Since excipients are absent in the formulation, a placebo solution was not prepared. The accelerated degradation studies conducted were the following:

a) **Acidic degradation:** 20mg of Doripenem powder for injection was dissolved in 20ml water in a 100ml volumetric flask and then added 20ml of 0.1 N HCl solution, heated at 50°C for 30 min. Cooled, neutralized the solution and made up to 100ml with water. The solution was centrifuged at 2500 rpm for 10min. 1ml of the supernatant solution was diluted to 10ml with the mobile phase.

b) **Alkaline degradation:** 20mg of Doripenem powder for injection was dissolved in 20ml water in a 100ml volumetric flask, then added 20ml of 0.1 N NaOH solution.
solution, after 3 min neutralized the solution and made up to 100 ml with water. The solution was centrifuged at 2500 rpm for 10 min. 1 ml of the supernatant solution was diluted to 10 ml with the mobile phase.

c) Oxidative degradation: 20 mg of Doripenem powder for injection was dissolved in 20 ml water, in a 100 ml volumetric flask then added 60 ml of 30% H2O2 solution, after 20 min, made up to 100 ml with water. The solution was centrifuged at 2500 rpm for 10 min. 1 ml of the supernatant solution was diluted to 10 ml with the mobile phase.

d) Thermal degradation: 20 mg of Doripenem powder for injection was dissolved in 20 ml water in a transparent glass bottle and kept at 45°C for 48 h. Transferred the contents into a 100 ml volumetric flask and made up with water. The solution was centrifuged at 2500 rpm for 10 min. 1 ml of the supernatant solution was diluted to 10 ml with the mobile phase.

e) UV degradation: 20 mg of Doripenem powder for injection was exposed to sunlight for 48 h. Then it was dissolved in water and made up to 100 ml with water. The solution was centrifuged at 2500 rpm for 10 min. 1 ml of the supernatant solution was diluted to 10 ml with the mobile phase.

Linearity

Doripenem reference solutions were prepared at concentrations of 10, 20, 30, 40, 50 and 100 µg/ml. Standard plots were constructed and linearity was evaluated statistically by linear regression analysis that was calculated by least-squares regression.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). In order to evaluate the repeatability of the methods, six sample solutions were determined during the same day for three concentrations of Doripenem. Intermediate precision was studied by comparing the assays performed on two different days.

Accuracy

The accuracy was determined by the recovery of known amounts of Doripenem reference standard added to the samples. The results were expressed as the percentage of Doripenem reference standard recovered from the sample. All solutions were prepared in triplicate and assayed.

Limit of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ parameters were determined from the regression equation of Doripenem: LOD = 3.3 SY/α, LOQ = 10 SY/α; where SY is the standard error and α is the slope of the corresponding calibration curve.

Robustness

The robustness of the procedure was evaluated after changing the following parameters: the composition of the mobile phase (content of acetonitrile in the range %); the mobile phase flow rate (0.6-0.8 ml/min) and temperature (25 ± 2°C). For each parameter change its influence on the retention time, resolution, peak (height, area and width) was evaluated.

RESULTS AND DISCUSSION

Method development

The chromatographic conditions were optimized with a view to develop a stability indicating assay method. The experimental studies revealed that the column, Phenomenex C18 (250 × 4.6 mm, 5 µm) was most suitable, since it produced best chromatographic performance and acceptable peak characteristics including high resolution and very good sensitivity. The Chromatographic conditions finally comprised of a mobile phase consisting of a mixture of 0.012 M ammonium acetate solution (pH 6.73) and Acetonitrile in the ratio of (85:15) at a flow rate of 0.5 ml/min using Phenomenex C18 column (250 × 4.6 mm, 5 µm) and detection was carried out at 295 nm.

Calibration curve

Under the above mentioned experimental conditions, a linear relationship was established by plotting Doripenem concentrations against the corresponding peak areas. The response was linear over the range of 10 to 100 µg/ml of Doripenem with a correlation coefficient, slope and intercept of 0.99944, 48.36 and -25.02 respectively. Under the applied chromatographic conditions, the LOD and LOQ of Doripenem were 0.5 and 1.5 µg/ml respectively.

Validation of the method

Precision

The intraday and inter-day precision values were calculated for three concentrations of Doripenem. The RSD values were less than 1%, demonstrating that the method was precise.

Accuracy

The accuracy of the method was established by recovery studies. Results indicate that the individual recovery of Doripenem ranges from 99.42% to 101.62% with mean recovery of 100.36% and %RSD of 0.45%. The recovery of the Doripenem by proposed method is satisfactory as %RSD is not more than ±2.0% and mean recovery between 98.0 – 102.0%.

Ruggedness and Robustness

The method robustness and ruggedness was determined by changing the following parameters: composition of the mobile phase, mobile phase flow rate in the range of 0.4-0.8 ml/min, temperature in the range 23-27°C and pH in the range 6.3-7.0. With the deliberate aforementioned change in parameter, the effects on the retention time and peak resolution, peak shape and peak area (height and width) were evaluated. No significant changes in the resolution and shapes of the peak and retention time.
were observed when temperature and flow rate were modified. Modifications of the composition of the mobile phase by changing the organic-to-inorganic ratio resulted in the essential changes of retention time and resolution in the determination of Doripenem.

**Analysis of marketed formulation**

The drug content was found to be 99.98% with %RSD of 0.32. It was noted that no degradation had occurred in the marketed formulation that were analyzed by this method. The low RSD value indicated the suitability of this method for routine analysis of Doripenem in pharmaceutical dosage forms.

**Stability indicating property**

The chromatogram of no stress treatment sample (as control) showed no additional peak (Fig 2). The chromatogram of acid degraded sample showed 87% degradation in the peak intensity at retention time of 7.13 min (Fig 3). The chromatogram of alkali degraded sample showed 100% degradation and shift of peak at retention times of 4.65 min for the degraded component (Fig 4). The chromatogram of H\textsubscript{2}O\textsubscript{2} degraded sample showed a 40% decrease in intensity of peak at 7.13 min and an additional small peak at retention time of 5.5 min corresponding to degraded component (Fig 5). There was no decrease in intensity of peak at 7.13 min in photo degraded sample (Fig 6) and thermal degraded sample (Fig 7). Thus Doripenem was found to be stable to dry heat and sunlight. In each forced degradation samples where additional peaks were observed, the response of the drug was changing from the initial control sample. This indicates that the drug is susceptible to acid-base hydrolysis degradation and H\textsubscript{2}O\textsubscript{2} degradation. The lower retention times of the degraded components indicated that they were more polar than the analyte itself (Table 1).

**Detection of the related impurities**

The sample solution showed no additional peaks other than the principal peak. Hence related impurities are not present in the market sample.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Degradation</th>
<th>Additional peaks</th>
<th>Peak purity\textsuperscript{a}</th>
<th>Tail peak\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid 0.1N HCl, heated at 50°C for 30 min</td>
<td>87%</td>
<td>-</td>
<td>99.8</td>
<td>1.13</td>
</tr>
<tr>
<td>Base 0.1N NaOH at room temp. for 3 min</td>
<td>100%</td>
<td>4.65 min</td>
<td>98.8</td>
<td>1.11</td>
</tr>
<tr>
<td>30% H\textsubscript{2}O\textsubscript{2} heated for 20 min</td>
<td>40%</td>
<td>5.50 min</td>
<td>98.9</td>
<td>1.11</td>
</tr>
<tr>
<td>Thermal, Heating at 45°C for 48 h</td>
<td>0%</td>
<td>-</td>
<td>99.8</td>
<td>1.13</td>
</tr>
<tr>
<td>Exposed to sunlight for 48 h</td>
<td>0%</td>
<td>-</td>
<td>99.5</td>
<td>1.20</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Peak purity values in the range of 98-100 indicate a homogeneous peak; \textsuperscript{b}Tail peak <1.50 indicates symmetry of peak.
CONCLUSION

The developed HPLC technique is simple, precise, accurate and stability indicating. As the carbapenems are sensitive to degradation, selectivity is an important validation parameter. Statistical analysis proves that the method is reproducible and selective for the analysis of Doripenem in pharmaceutical dosage forms. It can be used to determine the purity of the drug available from various sources. As the method separates the drug from its degradation products, under all stress conditions using HCl, NaOH, H$_2$O$_2$, heat and UV light, it can be employed as a stability indicating one.

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REFERENCES


15. ICH Guidance on analytical method validation, International convention on quality for the pharmaceutical industry, Toronto, Canada, 2002, 8-10.


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