Stability-Indicating RP-HPLC Method for Analysis of the Antibiotic Doripenem in Pharmaceutical Formulation—Comparison to UV Spectrophotometry and Microbiological Assay

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Summary. A stability-indicating liquid chromatographic (LC) method with UV detection was developed for the determination of doripenem in the marketed formulation (Doribax® 500 mg, powder for injection). A forced degradation study was conducted according to available guidelines and main references. Thermal, oxidizing, acidic and basic stress conditions were assayed to show the stability-indicating power of the method. Chromatographic separation was achieved using an isocratic elution method in a reversed-phase system using a mobile phase prepared from phosphate buffer and acetonitrile. Extensive degradation was observed under thermal, oxidative and basic treatment, and the products formed were detected without interference in the analysis of doripenem. To verify the efficiency of chromatographic run, the system suitability was studied. The theoretical plates ($N = 5498.3$) and tailing factor ($tf = 0.951$) were constant during repeated injections. The retention time of doripenem was 7.35 min and the method was validated within the concentration range 5–50 $\mu$g mL$^{-1}$ ($r = 0.999$). Adequate results were obtained that indicate repeatability (RSD % = 1.03–1.37), inter-day precision (RSD % = 0.51) and accuracy. In comparison to spectrophotometric and microbiological methods, statistical analysis showed no significant difference between the obtained results. The proposed method was successfully applied to doripenem quantification, showing it is applicable to determine the antibiotic in the presence of degradation products and also that is a reliable method for routine analysis.

Key Words: doripenem, RP-HPLC method, stability-indicating assay
Introduction

Doripenem (Doribax®, Ortho-McNeal Pharmaceuticals, Raritan, NJ, USA) (Fig. 1), (+)-(4R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-4-methyl-7-oxo-3-[[3S,5S]-5-[(sulfamoylamino)-methyl]-3-pyrrolidinyl]thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, is a broad-spectrum carbapenem antibiotic with antibacterial activity against a broad range of Gram-positive and Gram-negative bacteria, as well as against a variety of anaerobes [1–3]. It has been recently approved in the European Union and the United States for clinical use in complicated intra-abdominal and urinary tract infections, joining imipenem, meropenem and ertapenem [4, 5]. In comparison to other carbapenems, it has equal or greater activity than meropenem and imipenem against β-lactam-nonsusceptible Enterobacteriaceae, including strains with extended-spectrum β-lactamases (ESBLs) [2, 6]. Doripenem was also found to be the most active carbapenem against Pseudomonas aeruginosa [1, 5, 7]. Many studies have been published describing several aspects of antibacterial activity, resistance and clinical efficacy of doripenem, which illustrates its increasing clinical use [2, 6, 8–11].

![Fig. 1. Chemical structure of doripenem](image)

This crescent clinical application of doripenem promotes the need to study the main characteristics that could influence its quality during handling and storage and to supply analytical methods that can be used in routine quality control. The literature describes few studies regarding qualitative and quantitative methods to identify and quantify doripenem. These works describe stability evaluation in infusion solutions applying chromatographic analysis by liquid chromatography (LC) using isocratic elution to determine the antibiotic [12, 13], and also drug determination by derivative UV spectrophotometry [14]. Other studies include the quantitation of doripenem in biological fluids [15, 16]. Sutherland and Nicolau (2007) have described the determination of the drug in human serum by LC using meropenem as an internal standard [16]. Considering
the official compendia, there is no monograph containing methods to characterize or quantify doripenem. Such methods could offer official parameters to guarantee the validity of the assay.

The present study describes the development and validation of an RP-HPLC based method for quantitation of doripenem. This assay was compared to spectrophotometric and microbiological methods, which were previously validated with the same purpose. The LC method was studied following the official guidelines [17, 18], evaluating the main parameters and the procedures that can be applied to consider a stability-indicating assay. According to an FDA guidance document [19], a stability-indicating method accurately measures the active ingredients without interference from degradation products, process impurities, excipients or other potential impurities.

### Experimental

#### Chemicals

Doripenem reference standard (99.4%) and Doribax® powder for injection (570 mg of doripenem monohydrate containing 500 mg of doripenem as anhydrous basis) were purchased from AK Scientific, Inc. (Mountain View, USA). All chemicals were of analytical grade, and all solvents were of HPLC grade. Acetonitrile was purchased from Tedia (Fairfield, OH, USA). Monobasic sodium phosphate was purchased from Synth (São Paulo, Brazil). Purified water was prepared using Milli-Q Plus® (Millipore, Bedford, USA).

#### Apparatus

The LC method was performed on a Prominence Liquid Chromatograph Shimadzu, equipped with an LC-20AT pump, SIL-20A auto sampler, SPD-20AT PDA detector and CTO-20A column oven (Shimadzu, Kioto, Japan). LC Solution V. 1.24 SP1 system software was used to control the equipment and to calculate data and responses from the LC system. To perform the thermal degradation, a dry air oven (Nova Ética®, São Paulo, Brazil) was used.

#### Chromatographic Conditions

The method was conducted using a reverse-phase technique. Doripenem was eluted isocratically with a flow rate of 1.0 mL min⁻¹ using a mobile phase consisting of 10 mM monobasic phosphate buffer (pH 4.8) and acetonitrile (96:04; v/v). The wavelength of the UV–vis detector was set to
298 nm. The mobile phase was prepared daily, filtered through a 0.45 μm membrane filter (Millipore) and sonicated before use. A Nucleosil 100-5 C18 column (150 mm, 4.6 mm i.d., 5 mm particle size) (Macherey-Nagel, Düren, Germany) was used. The HPLC system was operated at 25 ± 1 °C.

Sample Preparation to Analysis

The doripenem reference standard was accurately weighed and dissolved in a 100 mL volumetric flask with purified water to obtain a concentration of 100.0 μg mL⁻¹. This solution was diluted in purified water to yield a final concentration of 20.0 μg mL⁻¹.

For the powder for injection, 10 mg of doripenem was transferred to a 100 mL volumetric flask and dissolved in purified water for a concentration of 100 μg mL⁻¹. An aliquot of this solution was diluted with the same solvent until the concentration was 20 μg mL⁻¹ of doripenem. Both sample and standard solutions were filtered through a 0.45 μm membrane filter (Millipore) before the injection.

System Suitability

The system suitability test was performed to ensure that the chromatographic assay was suitable to the analysis intended. A standard solution containing 20 μg mL⁻¹ of doripenem was injected in triplicate. Chromatographic parameters including peak area, retention time, theoretical plates and tailing factor were measured, and the relative standard deviation (RSD) for each parameter was determined.

Validation Procedure

The chromatographic method was validated by evaluation of the analytical parameters including specificity, linearity, precision, accuracy and robustness [17, 18]. The stability-indicating capability was determined by forced degradation conditions, including testing heat, oxidation and acid and basic degradation [20].

Specificity

Considering the absence of excipients in the formulation, a placebo solution was not prepared. The accelerated degradation conditions applied were the following: heat, acid, basic and oxidant media. All results were compared to
a reference and sample solution analysis, prepared the same day. The specific conditions performed during the study are described below:

(a) Heat: A solution of doripenem powder for injection was prepared in water at concentration of 1.0 mg mL\(^{-1}\) and stored in transparent glass in a stove at 45 °C for 48 h. An aliquot of this solution was diluted with the same solvent for a concentration of 20 µg mL\(^{-1}\) of doripenem.

(b) Oxidation: Doripenem powder for injection was dissolved directly in hydrogen peroxide solution (30%) in volumetric flask at concentration of 100 µg mL\(^{-1}\) at room temperature. After 30 min, an aliquot was diluted with water for a doripenem concentration of 20 µg mL\(^{-1}\). A control solution containing hydrogen peroxide was also prepared.

(c) Acid and alkaline hydrolysis: Doripenem powder for injection was transferred to volumetric flask and dissolved in either a 0.1 N HCl solution for acidic degradation acid or a 0.1 N NaOH solution for alkaline degradation, achieving a final concentration of 1 mg mL\(^{-1}\). After 3 min (alkaline degradation) and 1 h (acid degradation), one aliquot of the solution was neutralized and diluted with water until a final concentration of 20 µg mL\(^{-1}\) was reached. This solution was analyzed.

**Linearity**

Doripenem reference solutions were prepared in triplicate at concentrations of 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 µg mL\(^{-1}\). Standard plots were constructed and linearity was evaluated statistically by linear regression analysis that was calculated by least-squares regression and analysis of variance (ANOVA).

**Precision**

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Six sample solutions of doripenem powder for injection were prepared at 20 µg mL\(^{-1}\) on three different days. The analyses were done in triplicate and the results were expressed as an RSD of the analytical measurements.

**Accuracy**

The accuracy was determined by the recovery of known amounts of the doripenem reference standard added to the samples. The added levels were
20%, 40% and 60% of the sample concentration (20 μg mL$^{-1}$). The results were expressed as the percentage of doripenem reference standard recovered from the sample. All solutions were prepared in triplicate and assayed.

**Robustness**

Robustness testing was performed to evaluate the susceptibility of measurements under deliberate variations in selected analytical conditions. Four factors were assayed: pH of aqueous phase, temperature, proportion of mobile phase and flow rate. The variations are illustrated in *Table I*. During robustness testing each condition was assayed separately, with all other conditions held constant at the selected values.

*Table I. Factors used during system suitability determination*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Reference</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH of aqueous phase</td>
<td>4.8</td>
<td>4.6; 5.0</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25</td>
<td>23; 27</td>
</tr>
<tr>
<td>Concentration of organic solvent (%)</td>
<td>4.0</td>
<td>2.0; 6.0</td>
</tr>
<tr>
<td>Flow rate (mL min$^{-1}$)</td>
<td>1.0</td>
<td>0.8; 1.2</td>
</tr>
</tbody>
</table>

**Stability of Analytical Solutions**

The analytical solutions were evaluated in order to verify their behavior in usual analysis conditions. Doripenem powder for injection was prepared in purified water and in the mobile phase at concentration of 20 μg mL$^{-1}$. Triplicate samples of each solution were stored at room temperature (25 °C) and at 4 °C. After 24 h, both solutions were analyzed and the chromatographic pattern was compared to freshly prepared sample and standard solutions. An RSD (%) was calculated during these stability studies.

**UV Spectrophotometric Method**

The UV spectrophotometric method was performed on a Lambda 35 UV/vis Spectrophotometer Perkin Elmer (Norwalk, CT, USA) at 298 nm and using 1 cm quartz cells. For quantitative analysis, six sample solutions of doripenem powder for injection were prepared on three different days. A quantity equivalent to 10 mg of doripenem was transferred to a 100 mL volumetric flask and dissolved with distilled water to obtain a final concen-
tration of 100 μg mL\(^{-1}\). An aliquot of this solution was diluted with the same solvent until the concentration of 20 μg mL\(^{-1}\). The same procedure was done for reference standard.

**Microbiological Assay—Cylinder-plate Agar Diffusion**

The strain of *Micrococcus luteus* ATCC 9341 obtained from INCQS (Rio de Janeiro, Brazil) was cultivated after reconstitution and maintained in medium number 1 (Merck, Darmstadt, Germany). The microorganism standardization was prepared according to the procedure described in the Brazilian Pharmacopeia [21] and USP 31 [18] and for the antibiotics microbiological assays. The inoculum concentration was 2.0%. The bioassay described here followed the 3 × 3 parallel line assay design (3 doses of standard and 3 doses of sample in each plate), with 6 plates for each assay (two assays a day for three days), according to the Brazilian and European Pharmacopoeias [21, 22].

**Results and Discussion**

A simple, rapid and practical analytical procedure by LC was developed and validated for the determination of doripenem in a pharmaceutical formulation. According to ICH, the analytical procedure refers to the way of performing the analysis, describing in detail the steps necessary to perform each analytical test [17]. The goal of any analytical method is to produce analytical results that reflect the content of the samples with an acceptable standard of accuracy [23]. To show the capability to determine the drug in the presence of degradation products, a forced degradation study was performed and different degradation conditions were applied [20, 24].

**Method Development**

In the development of analytical assays, the best conditions must be chosen, including speed, reliability and reproducibility. LC still remains a method of choice, as it is able to separate quite complicated mixtures of low and high molecular weight compounds, as well as different polarities and acid-base properties in various matrices [24]. HPLC methods should be able to separate, detect and quantify the various drug-related degradants that can form upon storage or manufacturing [23]. In this work, the analytical parameters were studied to demonstrate that the assay is reliable for quantification of the drug. Knowledge of the UV absorption spectra of
doripenem and the relation solubility helped in the initial development of the assay. From the data about the absorption spectrum of meropenem [26, 27], the UV spectra of doripenem was performed in methanol, acetonitrile and water. In all solvents, maximal absorption was verified at 298 nm. Considering the environmental aspects, water was chosen to prepare the samples. We focused on the composition of the mobile phase in the preliminary evaluation of chromatographic profile and system suitability. The absence of buffer was intentional in the initial experiments, testing water–methanol or water–acetonitrile in different proportions. The results indicated a larger tailing peak for doripenem, which was not due to variability in inter-assays. Using phosphate buffer, the peak presented better symmetry and adjusting the proportion with organic solvent it was possible to achieve rapid analysis. The run time of the method was 10 min, allowing routine quality control of the product. Another preliminary study included the selection of the analysis concentration, which allowed simultaneous impurity detection. However, high concentrations of doripenem were injected, and no impurities were detected.

System Suitability

In pharmaceutical analysis (quality control), the suitability tests are focused on the analytical instrument and method. The analytical results are only valid if the defined system suitability criteria are fulfilled [28]. In this work, the experimental results (Table II) indicated that the chromatographic system was suitable for the intended analysis. The retention time for doripenem LC analysis was 7.35 min. The RSD values calculated for the peak area and retention time were 0.84% and 0.51%, respectively, indicating the reproducibility for these parameters. The tailing factor observed was 0.95 with an RSD of 0.31%, showing the peak symmetry. Theoretical plates of chromatographic separation were 5498.31 with an RSD of 1.93%.

Table II. Results from system suitability determination

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak area</td>
<td>641718.6</td>
<td>0.84</td>
</tr>
<tr>
<td>Retention time</td>
<td>7.35</td>
<td>0.51</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>5498.3</td>
<td>1.93</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.951</td>
<td>0.30</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean of three replicates.
Doripenem is marketed in a pure form, without excipients. Thus, the specificity was assayed in forced degradation experiments. According to FDA guidelines, stress testing of a drug substance can aid identification of likely degradation products, which can help to establish degradation pathways and reveal the intrinsic stability of the molecule [19]. Also, the demonstration of specificity and the ability of the method to monitor a change in the chemical properties of the drug over time invariably calls for a forced degradation (stress testing) study to be done on the drug substance and drug product [24]. A literature survey was done to determine the degradation of other carbapenems, such as meropenem and ertapenem [29–31]. For these antibiotics, thermal and alkaline degradation were the main factors described. To the best of our knowledge, photodegradation has not been mentioned or evaluated, indicating that the carbapenems are stable to photolytic degradation. In this work, the conditions tested were the following: heat, alkaline hydrolysis, acid hydrolysis and oxidation. The LC chromatograms for doripenem standard and sample are shown in Fig. 2. 

Fig. 2. LC chromatograms of doripenem standard solution (a) and sample solution sample (b), both at concentration of 20 \( \mu \text{g mL}^{-1} \)
Fig. 3. LC chromatograms of doripenem sample solution and degraded samples.
(a) Doripenem standard solution 20 μg mL⁻¹; (b) doripenem sample solution after thermal degradation (45 °C, 48 h); (c) doripenem sample solution after basic degradation (NaOH 0.1 N, 3 min); (d) doripenem sample solution after acid degradation (HCl 0.1 N, 3 min); (e) doripenem sample solution after oxidative degradation (H₂O₂ 10%, 10 min)
The drug was detected at 7.35 min. Fig. 3 shows the chromatograms of doripenem for specificity test. Under thermal degradation, it can be seen a more polar product at 2.50 min (Fig. 3b). In alkaline condition, the drug was completely degraded (Fig. 3c), whereas no degradation products were observed after treatment with acid (Fig. 3d). At alkaline decomposition, a peak at 2.6 min can be also observed, with the same peak profile of the peak observed for thermal decomposition. The extensive degradation with NaOH was also observed with meropenem, another carbapenem, and the product formed in this case was a β-lactam ring-opened derivative, detected at 2–3 min in the studied conditions [26, 29]. This product was isolated and identified by NMR and ESI–MS [29]. The same product was verified by Mendez et al. after thermal decomposition of meropenem [26]. Thus, based on the findings and the literature survey, it can be suggested that the compound detected after thermal and alkaline degradation of doripenem is the β-lactam ring-opened derivative. In oxidative conditions (Fig. 3e), it was observed 18.10% of drug degradation and peaks at 2.91 and 3.64 min. Under this condition, doripenem exhibits a symmetrical peak which is well resolved from the degradation products and with a resolution of 10.74 from the nearest eluting peak. Chromatograms of the degraded samples allowed us to conclude that degradation products do not interfere with the analysis of doripenem, indicating that the developed LC method was selective for the determination of the antibiotic in the pharmaceutical formulation.

**Linearity**

To assess linearity, three standard curves for doripenem were constructed by plotting concentration of drug (x) versus peak area (y). Over the concentration range of 5–50 μg mL⁻¹, the slope and the intercept obtained were 28,967 and 4279, respectively, and the correlation coefficient was 0.9999, indicating an excellent correlation between the parameters cited above. The statistical results obtained from ANOVA showed that the regression equation was linear ($F_{\text{calculated}} = 1820.18 > F_{\text{critical}} = 4.96; p = 0.05$) with no deviation from linearity ($F_{\text{calculated}} = 0.520 < F_{\text{critical}} = 3.71; p = 0.05$).

**Precision**

The reproducibility of the method was determined by performing six replicate analyses in an independent sample matrix. The results are shown in Table III. In intra-assay precision, the RSD value for peak area of doripenem was between 99.48% and 100.45%. The intermediate precision was determined by analyses in three different days. The low values of RSD
for both the repeatability and the intermediate precision demonstrate the good precision of the method proposed.

Table III. Results from precision determination of the LC assay for doripenem powder for injection (Doribax®, 500 mg)

<table>
<thead>
<tr>
<th>Day</th>
<th>Label claim (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>99.69</td>
<td>1.17</td>
</tr>
<tr>
<td>Day 2</td>
<td>100.45</td>
<td>1.03</td>
</tr>
<tr>
<td>Day 3</td>
<td>99.48</td>
<td>1.37</td>
</tr>
<tr>
<td>Inter-day</td>
<td>99.87</td>
<td>0.51</td>
</tr>
</tbody>
</table>

*aMean of six amounts, assayed in triplicate.

Accuracy

The accuracy of the method was studied by applying the recovery test, which was done by the analysis of reference standard added in sample solution. Calculation of accuracy was carried out as the percentage of drug recovered from the real samples. The results are summarized in Table IV. Mean recovery for doripenem reference from the sample was between 99.29 and 101.16 (n = 3), indicating that the developed method was accurate for the determination of the drug.

Table IV. Results from accuracy evaluation of the LC method for doripenem powder for injection

<table>
<thead>
<tr>
<th>Level (%)</th>
<th>Amount of drug added (μg mL⁻¹)</th>
<th>Amount of drug found (μg mL⁻¹)</th>
<th>Mean of recovery (%)a</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>4.0</td>
<td>4.04</td>
<td>101.16</td>
<td>1.76</td>
</tr>
<tr>
<td>40</td>
<td>8.0</td>
<td>4.03</td>
<td>100.84</td>
<td>0.92</td>
</tr>
<tr>
<td>60</td>
<td>12.0</td>
<td>3.97</td>
<td>99.29</td>
<td>1.15</td>
</tr>
</tbody>
</table>

*aMean of three amounts, assayed in triplicate.

Robustness

Robustness is the ability to reproduce the analytical method under different circumstances without the occurrence of unexpected differences in the
obtained results [32]. By evaluating the results obtained from the analysis performed under all the deliberately varied chromatographic conditions, the LC method developed indicated good performance, demonstrating to be a robust and reliable method in the determination of the drug. The chromatographic pattern was maintained with conditions including pH, flow rate and temperature, with small changes in the retention time. A major variation in the retention time occurred when the mobile phase was modified. Despite retention time, the quantitation of doripenem was maintained in all conditions, with low values of RSD. The results are shown in Table V.

Table V. Results from robustness testing of the LC method for doripenem powder for injection

<table>
<thead>
<tr>
<th>Robustness condition</th>
<th>Nominal conditiona</th>
<th>pH of aqueous</th>
<th>Concentration of organic solvent (%)</th>
<th>Temperature (°C)</th>
<th>Flow rate (mL min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.6</td>
<td>5.0</td>
<td>06</td>
<td>23</td>
<td>0.8</td>
</tr>
<tr>
<td>Amount (%)</td>
<td>100.14</td>
<td>100.79</td>
<td>100.61</td>
<td>100.40</td>
<td>100.19</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.22</td>
<td>1.27</td>
<td>1.48</td>
<td>1.15</td>
<td>1.04</td>
</tr>
<tr>
<td>Retention time</td>
<td>7.34</td>
<td>7.84</td>
<td>7.15</td>
<td>4.53</td>
<td>7.65</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>5659.7</td>
<td>4552</td>
<td>4398.5</td>
<td>4542</td>
<td>5262.7</td>
</tr>
</tbody>
</table>

aNominal condition: pH 4.8; flow rate 1.0 mL min; concentration of organic solvent 4.0%; temperature 25 °C.

Solution Stability

The results obtained indicate that the analytical solutions, prepared according to the described procedure, present adequate stability at 4 °C as both retention time and peak area were not modified. The RSD obtained was less than 2.0% and no significant degradation was observed during the time (24 h) of storage. The analytical solution was prepared in the mobile phase and stored at 4 °C; the results were also adequate and no modification was observed in chromatographic pattern. This result is important in improving reliability in the chromatographic run.
Method Application

The validated LC method was successfully applied to the assay of doripenem in a commercially available sample (Doribax®). In absence of a reference method, a comparative study is not possible, although all results presented illustrate an excellent performance of the assay according to the official guidelines. The chromatographic profile obtained is typical for drug analysis, with a clear chromatogram and with a rapid run. An important advantage was the low quantity (4%) of organic solvent necessary to perform the method, once the sample preparation was done in purified water. For the stress stability testing, the method was applied to determine doripenem in the presence of degradation products. The results clearly indicated that the drug was unstable under accelerated storage conditions and there was no interference from degradation products with analysis of doripenem. For future studies, the presented data could be useful to understand the stability of this drug, the main degradation products and the decomposition pathway involved.

Comparison of Methods

The LC method was statistically compared using ANOVA with previously validated spectrophotometric and microbiological methods. The experimental values obtained for the determination of doripenem results by each method are described in Table VI. It was found that the amounts of

<table>
<thead>
<tr>
<th>Method</th>
<th>HPLC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>UV spectrophotometry&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Microbiological assay&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>99.69 (498.45, 1.17)</td>
<td>99.73 (498.65, 1.22)</td>
<td>102.59 (512.95, 0.02)</td>
</tr>
<tr>
<td>Day 2</td>
<td>100.45 (502.25, 1.03)</td>
<td>101.98 (509.90, 0.96)</td>
<td>100.68 (503.40, 2.12)</td>
</tr>
<tr>
<td>Day 3</td>
<td>99.48 (497.25, 1.37)</td>
<td>102.41 (512.05, 0.63)</td>
<td>101.97 (509.85, 0.50)</td>
</tr>
<tr>
<td>Mean (inter-day precision)</td>
<td>99.87 (499.35)</td>
<td>101.37 (506.85)</td>
<td>101.75 (508.75)</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.51</td>
<td>1.41</td>
<td>0.95</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean of six amounts, assayed in triplicate.
<sup>b</sup>Mean of twelve replications (two assays of six plates a day).
doripenem determined using each method were not statistically different ($F_{\text{calc}} = 2.69 < F_{\text{tab}} = 5.14, p < 0.05$). It means that the methods could be used for the same purpose.

**Conclusion**

A simple, fast and reliable stability-indicating RP-HPLC method for quantitative analysis of doripenem was developed and validated. The drug was easily separated and quickly detected by a chromatographic technique. The system suitability illustrates good performance and reproducibility of analysis. The degradation products formed during the stress testing were well separated from the drug, indicating that the method is capable of indicating stability. In addition, the simple sample preparation procedure is adequate for routine quality control. Comparing to UV spectrophotometry and microbiological assay, it was possible to state that the proposed method is equivalent for determination of doripenem in powder for injection, representing reliable alternatives for this analysis.

**References**

[22] European Pharmacopoeia, Main volume 5.0, 5th ed., Council of Europe, Strasbourg, 2005

Accepted by DA