INTRODUCTION
As a medication, caspofungin attacks fungus. Some dangerous fungi, such as Candida albicans and Aspergillus fumigatus, rely on 1, 3-β-D-glucan production to build their structural cell walls, which it hinders. It stops pathogenic fungi’s membrane fractions from transferring glucose enzymatically from UDP glucose 1, 3-β-D-glucan.

The molecular weight of the compound is 1213.42 gm/mol and the empirical formula is C52H88N10O15.2C2H4O2 (Figure 1). A sterile, lyophilized medication intended for intravenous (IV) infusion, capsofungin acetate for injection was first licensed in 2001 by EMA Food and Drug Administration (FDA) in the United States. Literature has not published many high-performance liquid chromatography (HPLC) techniques for measuring caspofungin in plasma and other biological materials. To the best of our acquaintance, no stability-indicating HPLC method for parenteral dosage form caspofungin determination has been published.

MATERIAL AND METHODS
Caspofungin was obtained as a gift sample from Gift sample from Cipla Pvt Ltd, Mumbai. potassium dihydrogen phosphate, ammonium acetate, sodium hydroxide, ethanol, acetonitrile, water (HPLC grade), triethylamine, and acetic acid was procured from Shree Sadguru Hitech Pvt Ltd, Pune. The injection of casopgin (70 mg) was bought from Market.

Chromatographic Equipment and Conditions
The LC equipment used for method development and validation was a Thermo P4000 Quaternary pump, a UV 6000 PDA detector with CHROMQUEST software, an Ultrasonic cleaning power sonic420, a UV spectrophotometer (UV 3092, Lab India, Mumbai) and a pH meter (Thermo electron business Orion 2 star).

ABSTRACT
In order to more precisely and accurately estimate the antifungal medicine caspofungin and its parenteral dosage form, a new, straightforward, and cost-effective reverse-phase high-performance liquid chromatography (RP-HPLC) technique has been created. The accuracy, precision, linearity, specificity, and reproducibility of the method for the measurement of caspofungin in used equipment were determined during the present study’s validation according to the International Council of Harmonization (ICH) criteria. Elution was accomplished using a CHEMSIL ODS-C18 (5μm) column in isocratic mode with a mobile phase consisting of acetonitrile:ammonium acetate buffer at pH 4.8 (60:40%v/v). The mobile phase was pumped into the column at a flow rate of 1.0 mL/min, and the eluent was detected using a variable wavelength UV detector set at 210 nm. The column dimensions are 250 × 4.6 mm. The chromatogram for the optimized RP-HPLC method of caspofungin estimation had 28,99 theoretical plates (N) and a strong peak at a resolution time of 16,105 minutes. The thorough validation process led to the conclusion that the approach is stable and could be applied for the duration of the drug’s shelf life.

Keywords: RP-HPLC, ICH guidelines, Antifungal, Caspofungin.
Preparation of Standard Solution

About 10 mg of caspofungin were added to a 100 mL volumetric flask that was dry and clean. After adding approximately 70 mL of diluents, the mixture was thoroughly dissolved using sonication. The volume was increased to the necessary level by using the remaining solvent, i.e., stock solution.5,6

Preparation of Sample Solution

To make a clear solution, bring the conventional injectable vial containing 70 mg of caspofungin (as acetate) to room temperature. Then, add 14.10 mL of 0.9% sodium chloride and stir thoroughly. After diluting 10 mL of the solution with diluent to 50 mL, it to sonication for 5 minutes with occasional shaking, allowing it to cool at room temperature, and finally filtering it through a 0.45 µ nylon filter. About 1000 µg/mL is the concentration of caspofungin7,8

Analytical Method Validation

The following criteria were used to validate the new reverse-phase high-performance liquid chromatography (RP-HPLC) method: stability of analytical solutions, specificity, linearity, sensitivity, precision, and accuracy. International Council of Harmonization (ICH) recommendations for validation of analytical techniques were followed in the execution of validation.9

HPLC Method Validation

Linearity, LoDs, and LoQs

A final concentration of 5, 10, 15, 20, 25, and 10 µg/mL of caspofungin was achieved by diluting an appropriate amount from the stock solution. The next step was to capture the chromatogram. Draw a graph showing the relationship between concentration and the number of theoretical plates at each concentration. It was necessary to determine the peak area after injecting each level of solution into the chromatographic device. We determined the correlation coefficient after plotting peak area vs concentration.10

Precision

• Repeatability

Accurately measure out 10 mg of capsaicin. Next, fill a 100 mL clean and dry volumetric flask with the mixture. After adding 70 mL of diluent, sonicate it until it dissolves completely. Then, use the same solvent to adjust the volume. An extra milliliter of caspofungin from solution A was pipetted into a 10 mL volumetric flask and the remaining volume was diluted with diluents to the correct concentration. The area was determined using HPLC after each of five injections of the standard solution. The results showed that the percentage RSD for each of the five replicate injections was within the acceptable range.11

• Intermediate precision

Using distinct columns of the same dimensions, the method's intermediate precision – also denoted to as its ruggedness – was assessed on various days.

• Accuracy

The HPLC system was injected with standard solutions, as well as solutions with different levels of accuracy (50, 100, and 150%). Caspofungin individual and mean recovery values, as well as the amount detected and added, were also computed. The average recovery percentage of caspofungin was calculated.12

• Robustness

The HPLC system was injected with standard solutions, as well as solutions with different levels of accuracy (50, 100, and 150%). Caspofungin individual and mean recovery values, as well as the amount detected and added, were also computed. The average recovery percentage of caspofungin was calculated.

• Effect of variation of flow rate

Researchers looked at the effects of different flow rates to find out what they were. The flow rate varied between 0.8 and 1.2 mL/min. The technique flow rate and several flow rates were used to analyze a 10 ppm Caspofungin standard solution. Here is a rundown of the results. The above data analysis suggests that the strategy was significantly affected by the flow rate variance. Therefore, it implies that the method is stable regardless of ±10% variations in the flow rate. The strategy only holds up well in low-flow scenarios. Variation in flow rate was evaluated for its potential effects.13

• Specificity

HPLC system was filled with standard and sample solutions that had been prepared in accordance with test protocol. Chromatograms that were recorded are displayed in findings.14

System suitability

Caspofungin sample solution was injected into the HPLC system three times in accordance with the test protocol. The system suitability characteristics were evaluated using standard chromatograms calculated from three replicate injections using %RSD of retention times, tailing factor, theoretical plates, and peak regions.15,16

RESULT AND DISCUSSION

Method Development and Optimization

The standard chromatogram obtained is given in Figure 2, while the linearity graph is illustrated in Figure 3. The chromatographic details of caspofungin are given in Table 1.

The optimized method for caspofunginestimation showed a chromatogram with a sharp peak at a resolution time 16.124 minutes and had 2899 theoretical plates (N).

Linearity

The equation of the linearity graph for caspofungin was y = 5.58x + 2841, and the correlation coefficient was 0.999. Therefore, the concentration range of the technique is 5 to 25 µg/mL, and it is linear (Table 2).17
Accuracy (%Recovery)
A conventional addition approach was used to carry out the recovery experiment. Studies on accuracy were conducted at concentrations of 50, 100, and 150%. For example, 5, 10, and 15 µg/mL (Table 3).

The range of 99.2 to 100.2% was determined to represent the mean recoveries. The recovery outcome shows that the suggested approach is precise.

Intermediate Precision
The area of each injection was measured using an HPLC after five injections of standard solution. It was found that %RSD for each of the five injection replicates was within the allowed range. Based on the test procedure, the investigation was carried out by two analysts.

The assay value was discovered within the range of 97 to 103%, and the caspofungin RSD values are no greater than 2.0, indicating the precision of the procedure (Table 5).

Robustness
Effect of variation in flow rate
Researchers looked at the effects of different flow rates to find out what they were. The flow rate varied between 0.8 and 1.2 mL/min.

Variation in flow rate was evaluated for its potential effects. This is because the flow variation was within acceptable ranges for both the asymmetry and the retention time percent RSD. As a result, the allowed flow rate falls anywhere between 0.8 and 1.2 mL (Table 6).

Effect of variation of mobile phase composition
An experiment was conducted to determine the effects of changing the mobile phase ratio. The organic composition of the mobile phase was fine-tuned to within ±2% v/v.
Table 5: Results of caspofungin for intermediate precision (reproducibility)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>%&lt;i&gt;assay&lt;/i&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean*</td>
<td>99.13</td>
</tr>
<tr>
<td>SD</td>
<td>0.023</td>
</tr>
<tr>
<td>%RSD*</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Table 6: Results for caspofungin robustness by RP-HPLC

<table>
<thead>
<tr>
<th>Flow rate (mL/min)</th>
<th>Theoretical plates (N)</th>
<th>Robustness results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USP tailing</td>
<td>USP tailing</td>
</tr>
<tr>
<td>0.8</td>
<td>2948</td>
<td>1.53</td>
</tr>
<tr>
<td>1.0</td>
<td>2954</td>
<td>1.48</td>
</tr>
<tr>
<td>1.2</td>
<td>2978</td>
<td>1.48</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.0288</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td>1.93%</td>
</tr>
</tbody>
</table>

Table 7: Robustness results for caspofungin by RP-HPLC

<table>
<thead>
<tr>
<th>Mobile phase composition (v/v)</th>
<th>Theoretical plates (N)</th>
<th>Robustness results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USP tailing</td>
<td>USP tailing</td>
</tr>
<tr>
<td>Mobile phase +2%</td>
<td>2978</td>
<td>1.8</td>
</tr>
<tr>
<td>Mobile phase -2%</td>
<td>2948</td>
<td>1.9</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.0707</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td>0.382</td>
</tr>
</tbody>
</table>

Table 8: System suitability results for caspofungin by RP-HPLC

<table>
<thead>
<tr>
<th>Injection number</th>
<th>Concentration (µg/mL)</th>
<th>Theoretical plates (N)</th>
<th>Robustness results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP tailing</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>2952</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>2956</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>2950</td>
<td>1.4</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td>0.0057</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
</tbody>
</table>

The effects of varying the mobile phase’s composition were evaluated. Since asymmetry and retention duration percent %RSD fell within acceptable bounds for compositional variation in the mobile phase (Table 7).

Specificity

Both standard and sample chromatograms have roughly the same retention time. There was no placebo-related interference during the analyte’s retention period, indicating that the procedure was precise. At the retention period of the analyte peak, the blank chromatogram showed no signal. There was no interference from blanks during the analyte’s retention period. Thus, the approach is particular.

Limit of Detection and Limit of Quantification

The caspofungin limit of detection (LoD) (signal-to-noise ratio ≥ 3) was determined to be 4 ng/mL. It was discovered that limit of quantification (LoQ) was 12.5 ng/mL.

System Suitability

According to the system suitability studies, every parameter was found to be within acceptable bounds. An assay for the drug can be performed with the selected system conditions (Table 8).

CONCLUSION

A successful approach for estimating the dose form and bulk content of caspofungin was established using RP-HPLC. The method for determining the amount of capsaicin in utilized instruments was found to be accurate, exact, linear, specific, and repeatable. The current study was verified in accordance with the ICH guidelines. The thorough validation process led to the conclusion that the approach is stable and could be applied for the duration of the drug’s shelf life.

REFERENCES


