Development and application of Liquid Chromatographic method for determination of Caspofungin Acetate in sterile, lyophilized powder for Injection

Dr. L. Satyanarayana, Dr. Sumalatha Reddi
Department of Pharmaceutical Analysis,
Omega College of Pharmacy, Ghatkesar, Hyderabad, Telangana, India

ABSTRACT

Caspofungin is an antifungal agent of the novel echinocandin class. Caspofungin, the first inhibitor of fungal β-1,3 glucan synthesis to receive approval by the United States Food and Drug Administration, is effective for the treatment of mucosal and invasive candidiasis and invasive aspergillosis. It is also active in vitro and in animal models against a number of other filamentous and dimorphic endemic fungi and in animal models of Pneumocystis carinii infection. Caspofungin is a water-soluble amphipathic lipopeptide is a semisynthetic derivative of pneumocandin B0, a fermentation product of Glarea lozoyensis. Developing a accurate and precise analytical method for the estimation of caspofungin in a sterile, lyophilized product for intravenous (IV) infusion is very challenging, due to the formation of drug-drug and drug-excipient interactions. The present study demonstrates the applicability of chromatographic method to develop a new, sensitive, single HPLC method for the quantitative determination of antifungal agents in freeze dried powder for injection pharmaceutical dosage form. Chromatographic separation active pharmaceutical ingredient was achieved by using an isocratic elution at a flow rate of 1.0 mL/min on X-Terra RP-18 column (250mm×4.6 mm, 5μm particle size, 100Å pore size) at ambient temperature. The contents of the mobile phase were 3.48 gms of Di Potassium hydrogen ortho-phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 3.2 with dilute ortho-phosphoric acid (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) in a isocratic mode in the ratio of 30: 70 (v/v) of separation was used to resolve the Caspofungin. UV detection at 278 nm was employed to monitor the analytes. A linear response was observed for caspofungin over the concentration range 0.5–6 μg/mL. Limit of detection (LOD) and Limit of quantification (LOQ) for Caspofungin were found to be 0.001μg/mL, and 0.003μg/mL respectively.

Keywords: Caspofungin, Isocratic-HPLC, Casporan®, Lyophilized powder for injection

Introduction:

Casporan® is a sterile, lyophilized product for intravenous (IV) infusion that contains a semisynthetic lipopeptide (echinocandin) compound synthesized from a fermentation product of Glarea lozoyensis1-3. Casporan® is the first of a new class of antifungal drugs (echinocandins) that inhibit the synthesis of β (1,3)-D-glucan, an integral component of the fungal cell wall.4 Caspofungin acetate, the active ingredient of Casporan®, inhibits the synthesis of β (1,3)-D-glucan, an essential component of the cell wall of susceptible Aspergillus species and Candida species. β (1,3)-D-glucan is not present in mammalian cells5. Caspofungin has shown activity against Candida species and in regions of active cell growth of the hyphae of Aspergillus fumigates6-8. It is indicated for the treatment of invasive Aspergillus infections in patients who are refractory or intolerant of other therapies; treatment of candidemia and other Candida infections (intra-abdominal abscesses, peritonitis, pleural space); treatment of esophageal candidiasis; empirical treatment for presumed fungal infections in febrile neutropenic patients Casporan® (caspofungin acetate) is 1-[(4R,5S)-5-[2-aminoethyl]amino]N2 -(10,12-dimethyl-1-
oxotetradecyl)-4-hydroxy-L-ornithine]-5-[(3R)-3-
hydroxy-L-ornithine] pneumocandin B0 diacetate
(salt). Casporan® 50 mg also contains: 39 mg
sucrose, 26 mg mannitol, glacial acetic acid, and
sodium hydroxide. Casporan® 70 mg also contains 54
mg sucrose, 36 mg mannitol, glacial acetic acid, and
sodium hydroxide. Caspofungin acetate is a
hygroscopic, white to off-white powder. It is freely
soluble in water and methanol, and slightly soluble in
ethanol. The pH of a saturated aqueous solution of
caspofungin acetate is approximately 6.6. The
empirical formula is C\textsubscript{52}H\textsubscript{88}N\textsubscript{10}O\textsubscript{15}\textsubscript{•2C\textsubscript{2}H\textsubscript{4}O\textsubscript{2}} and the
formula weight is 1213.42\textsuperscript{9-10}. Casporan® 50 mg is a
white to off-white powder/cake for infusion in a vial
with a red aluminum band and a plastic cap supplied
as one single-use vial. Casporan® 70 mg is a white to
off-white powder/cake for infusion in a vial with a
yellow/orange aluminum band and a plastic cap.

**Figure-1: Chemical structures of Caspofungin**

A survey of literature has revealed only one analytical
method for the determination of caspofungin in
biological fluids. These include; high-performance
liquid chromatography (HPLC)\textsuperscript{11}. On the contrary, to
the best of our knowledge, there is no method
reporting the determination of Caspofungin in
pharmaceutical formulation. In this paper, we report
the simple precise and accurate RP-HPLC method for
the assay of caspofungin acetate for Intravenous (IV)
Infusion in sterile lyophilized powder for injection
dosage form. The new method is capable of
separating active ingredient present in the Intravenous
(IV) Infusion. Validation of the current method will
be performed according to the requirements of USP
for assay determination which include accuracy,
precision, selectivity, linearity and range.

**Experimental:**

**Chemicals and reagents:** Caspofungin was obtained
as kind gift sample from Gland Pharma Ltd,
Hyderabad. Potassium dihydrogen ortho-phosphate,
methanol, acetonitrile and ortho-phosphoric acid were
obtained from Merck, Mumbai, India. All the
solutions were prepared in Milli Q water (Millipore,
USA). Test samples composed of Casporan® 50 mg
lyophilized powder for intravenous administration
vial, Ranbaxy, India contains 50 mg of Caspofungin,
is obtained from local market.

**HPLC Instrumentation and Chromatographic
conditions:** Quantitative HPLC was performed on the
Waters Alliance 2695 Separations Module is a high
performance liquid chromatographic system with a
quaternary, low-pressure mixing pump and inline
vacuum degassing. Waters Alliance 2695 separation
module (Waters Corporation, Milford, USA)
equipped with 2489 UV/visible detector or 2998 PDA
detector with Empower 2 software was used for the
analysis. Flow rates from 50 uL/ min to 5 mL/min can
be generated for use with 2.1 mm ID columns and
larger. The auto-sampler has a maximum capacity of
120 vials (12x32, 2-mL) with programmable
temperature control from 4 to 40°C. A heated column
compartment provides temperatures from 5 degrees
above ambient to 65°C. The detector is a photodiode
array (model 2996) with a wavelength range of 190-
800 nm and sensitivity settings from 0.0001-2.0000
absorbance units The HPLC system was equipped
with a column compartment with temperature control
and an on-line degasser. X-Terra RP-C18 Column
(250x4.6 mm i.d; particle size 5 μm) was used for
separation of Caspofungin. The contents of the
mobile phase were 3.48 gms of Di Potassium
hydrogen ortho-phosphate (0.03M) in 1000 ml of
water and by adjusting the pH to 3.2 with dilute
ortho-phosphoric acid (mobile phase solvent-A) and
acetonitrile (mobile phase solvent-B) in a isocratic
mode in the ratio of 30: 70 (v/v) of separation was
used to resolute the Caspofungin. They were filtered
before use through a 0.45 μm membrane filter and
degassed by sonication. The flow was adjusted at 1.0
ml/min flow rate and 20 μL injection load volumes
were maintained. The eluted compounds were
monitored at 278 nm. The column oven temperature was
maintained at 25°C. Data acquisition, analysis,
and reporting were performed by Empower2 (Waters)
chromatography software.
Preparation of Solutions:

**Standard and stock solutions:** Standard solution of the active pharmaceutical ingredient was prepared in the following manner: Transfer 5 mg of caspofungin working standard into a 100 ml volumetric flask, dissolve and dilute with Acetonitrile and water in the ratio of 50:50 v/v as diluent. 5 ml of the resulting solution is further diluted up to 50 ml in volumetric flask with diluents. The resulting solution contains 5 μg/mL of caspofungin as working standard solutions. The prepared stock solutions were stored at 4 °C and protected from light.

**Preparation of the Sample solution:** Casporan® 50 mg is a white to off-white powder/cake for infusion in a vial with a red aluminum band and a plastic cap. Casporan® is a sterile, lyophilized product for intravenous (IV) infusion that contains a semisynthetic lipopeptide (echinocandin) compound synthesized from a fermentation product of Glarea lozoyensis. Casporan® 50 mg also contains: 39 mg sucrose, 26 mg mannitol, glacial acetic acid, and sodium hydroxide. The contents of 5 vials are emptied and their average weight was calculated. The vial contents were blended to a homogeneous powder and a quantity equivalent to 5 mg was weighed and transferred in to a 100-mL volumetric flask, extracted in diluent by sonication, and filtered through Whatman no. 41 filter paper. The filtrate (5 mL) was quantitatively transferred to a 50-mL volumetric flask, and solution was diluted to volume with the diluents. The resulting solution contains 5 μg/mL of caspofungin as working sample solutions. The prepared stock solutions were stored at 4 °C and protected from light.

**Solutions for validation study:**

**Calibration and Quality control samples:** Calibration standards (0.5–6 μg/mL of caspofungin were prepared from working standard solutions by appropriate dilution with Acetonitrile and water in the ratio of 50:50 v/v as diluents. Quality control (QC) samples for accuracy studies were prepared at three concentrations of the linearity range (4 μg/mL, 5 μg/mL and 6 μg/mL) for caspofungin were prepared from the standard solutions.

**Method Validation:** The developed chromatographic method was validated for selectivity, linearity, precision, accuracy, sensitivity, robustness and system suitability.

**Specificity:** The terms selectivity and specificity are often used interchangeably. The specificity of the developed LC method for quantification of active pharmaceutical ingredient was determined the presence of excipients present in pharmaceutical products. In specificity study, interference between drugs and excipients usually employed in lyophilized powder for injection was evaluated from the comparison of spectral purity obtained from the analysis for the standard solutions and sample solutions.

**System suitability:** The system suitability was assessed by six replicate analyses of the drugs at concentrations of 5 μg/mL for caspofungin. The acceptance criterion was ±2% for the RSD for the peak area and retention times for all four analytes. The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between peak and peaks of the other three analytes were defined.

**Linearity:** Linearity of the method was evaluated at seven equi-spaced concentration levels by diluting the standard solutions to give solutions over the ranges 10–120% target concentration for main analyte of interest. The calibration curves were constructed at seven concentrations between 0.5–6 μg/mL for caspofungin. These were injected in triplicate and the peak areas were inputted into a Microsoft Excel® spreadsheet program to plot calibration curves. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The peak areas of the analyte to concentration of analyte were used for plotting the linearity graph. The linearity data is reported in Table-3.

Table-3: Linearity Data for caspofungin

**Precision:** Precision was evaluated in terms of intra-day repeatability and inter-day reproducibility. The intra-day repeatability was investigated using six separate sample solutions prepared, as reported above, from the freshly reconstructed tablet formulations at 100% of the target level. Each solution was injected in triplicate and the peak areas obtained were used to calculate means and RSD% values. The inter-day reproducibility was, by preparing and analyzing in triplicate sample solutions from the reconstructed formulations at the same concentration level of intra-
day repeatability; the means and RSD% values were calculated from peak areas. (Table-4)

Table-4: Intra-day and inter-day precision data for for caspofungin

Accuracy: The accuracy of the method was determined by measuring the recovery of the drug by the method of standard additions. Quality control (QC) samples for accuracy studies were prepared at three concentrations of the linearity range (4 µg/ mL (80% dilution), 5 µg/ mL (100% dilution) and 6 µg/mL (120% dilution) for caspofungin were prepared from the standard solutions. Known amounts of 10% dilution of each drug corresponding to 80%, 100%, and 120% of the target test concentrations (0.5 µg/mL of caspofungin) were added to a placebo mixture to determine whether the excipients present in the formulation led to positive or negative interferences. Each set of additions was repeated three times at each level. Extraction sample preparation procedure is followed and assayed against qualified reference standard. The accuracy was expressed as the percentage of the analytes recovered by the assay. (Table-5)

Table-5: Accuracy: recovery data for caspofungin

Sensitivity: Limits of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio. The detection limit was determined as the lowest concentration level resulting in a peak area of three times the baseline noise. The limit of detection was determined by injecting progressively low concentrations of analyte of interest. The quantification limit was determined as the lowest concentration level that provided a peak area with signal-to-noise 10.

Robustness: To determine the robustness of the developed method, experimental conditions were deliberately changed and the relative standard deviation for replicate injections of caspofungin and the USP resolution factor between and the other two peaks were evaluated. The mobile phase flow rate was 1.0 mL/min. This was changed by ±0.2 units to 0.8 and 1.2 mL/min. The effect of stationary phase was studied by the use of LC columns from different batches at 25°C. The effect of buffer pH was studied at pH 3.0 and 3.4 (± 0.2 units). The chromatographic variations were evaluated for resolution between and the other three analytes in a system suitability solution with respect to retention time RT and % assay of drugs.

Table-6: Robustness data for caspofungin

Solution stability: To assess the solution stability, standard and test solutions were kept at 25 °C (laboratory temperature) for 24 h. These solutions were compared with freshly prepared standard and test solutions.

RESULTS AND DISCUSSION:

HPLC method development: The API solution of analyte of interest i.e., caspofungin was prepared in diluent at a concentration of 50ug/mL and scanned in UV-Visible spectrometer; and the caspofungin was found to have UV maxima at around 278 nm. Hence detection at 278 nm was selected for method development purpose. Some important parameters, pH of the mobile phase, concentration of the acid or buffer solution, percentage and type of the organic modifier, etc. were tested for a good chromatographic separation. The main analytical challenge during development of a new method was obtaining adequate retention of the polar compound caspofungin. Trials showed that acidic mobile phase with reverse phase column gives symmetric and sharp peaks. For this reason, potassium dihydrogen phosphate buffer with pH-3.2 was adjusted with o-phosphoric acid was preferred as acidic buffer solution. Acetonitrile and buffer n the ratio of 70:30 (v/v) was chosen as the organic modifier because it dissolves drugs very well. Mobile phase composition in isocratic mode at a flow rate of 1.0 mL per minute was observed for a good resolution. Then method was optimized to separate the active ingredient by changing to isocratic mode. The satisfactory chromatographic separation, with good peak shapes were achieved on X-Terra RP-18-C18 (250 × 4.6) mm with 5 µm particles, using the column temperature as maintained at 35°C and the detection was monitored at a wavelength of 278 nm. The injection volume was 20 µL. Acetonitrile and water in the ratio of 50:50 v/v) were used as diluent.

Method validation:

The developed method was validated, as described below, for the following parameters: system suitability, selectivity, linearity, precision, accuracy and LOD/LOQ.
Selectivity: Selectivity of the current method was demonstrated by good separation of the active ingredients. Furthermore, matrix components, e.g. excipients, do not interfere with the four analytes as they have no absorbance. The representative chromatogram (Fig. 5) of the fixed dosage form solution containing excipients showed no peak interfering with analytes; moreover the adjacent chromatographic peaks were separated with resolution factors >3. Overall, these data demonstrated that the excipients did not interfere with the active ingredients peaks, indicating selectivity of the method.

System suitability: The RSD values of peak area and retention time for the analytes are within 2% indicating the suitability of the system.

Figure-2: System suitability chromatogram of working standard solution contains 5 μg/mL of Caspofungin.

Table-2: Results of System suitability study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caspofungin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time</td>
<td>3.34 minutes</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>8355,229</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>1.281</td>
</tr>
<tr>
<td>HETP</td>
<td>2.99214x10^-5</td>
</tr>
<tr>
<td>USP plates/meter</td>
<td>33420.916</td>
</tr>
<tr>
<td>Resolution</td>
<td>3.544</td>
</tr>
<tr>
<td>Peak area</td>
<td>8492820</td>
</tr>
<tr>
<td>% of Peak area</td>
<td>99.98</td>
</tr>
</tbody>
</table>

Linearity and range: Seven concentration levels within 10–120% of the target concentration range for analytes were considered to study the linearity. The calibration curves were prepared by plotting the peak area of the drug to the respective concentrations, which were linear in the range of 0.5–6 μg/ mL for Caspofungin. Peak areas of the active ingredients and concentrations were subjected to least square linear regression analysis to calculate the calibration equations and correlation coefficients. The mean regression equations were found as y=1651731.671x+296419.5807 for caspofungin. The square of the correlation coefficient (r² > 0.999) demonstrated a significant correlation between the concentration of analytes and detector response. The results show that there is an excellent correlation between the peak area ratios and the concentrations of drugs in the range tested.
Table-3: Linearity data for the Casparan® lyophilized product for intravenous (IV) infusion.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Peak Area</th>
<th>Parameter</th>
<th>Caspofungin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 μg/mL</td>
<td>915070</td>
<td>Concentration Range</td>
<td>0.5-6 μg/mL</td>
</tr>
<tr>
<td>1 μg/mL</td>
<td>2098372</td>
<td>Regression equation</td>
<td>y=1651731.671x+296419.5807</td>
</tr>
<tr>
<td>2 μg/mL</td>
<td>3669520</td>
<td>Correlation Coefficient</td>
<td>0.999</td>
</tr>
<tr>
<td>3 μg/mL</td>
<td>5230975</td>
<td>0.95 Confidence interval</td>
<td>Lower-Limit-0.993/Upper Limit-1</td>
</tr>
<tr>
<td>4 μg/mL</td>
<td>7018803</td>
<td>0.95 Confidence interval</td>
<td>Lower-Limit-0.987/Upper Limit-1</td>
</tr>
<tr>
<td>5 μg/mL</td>
<td>8496198</td>
<td>Limit of Detection(LOD)</td>
<td>0.001 μg/mL</td>
</tr>
<tr>
<td>6 μg/mL</td>
<td>10158295</td>
<td>Limit of Quantification(LOQ)</td>
<td>0.003 μg/mL</td>
</tr>
</tbody>
</table>

Figure-3: Calibration Curve of Casparan® lyophilized product for intravenous (IV) infusion.

**Precision:** Precision of this method was determined by injecting the standard solution of the three analytes six times. The R.S.D. of peak area of six replicates was found to be less than 2%. The results obtained are shown in Table 4. In all instances the %RSD values were less than 2%.

Table-4: Intra-day and inter-day precision data for Caspofungin

<table>
<thead>
<tr>
<th>Precision data of Caspofungin</th>
<th>Inter-day precision</th>
<th>Intra-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte-conc. (5 μg/ml)</td>
<td>Retention time in min.</td>
<td>Peak Area</td>
</tr>
<tr>
<td>Caspofungin injection-1</td>
<td>3.159</td>
<td>8478697</td>
</tr>
<tr>
<td>Caspofungin injection-2</td>
<td>3.260</td>
<td>8505817</td>
</tr>
<tr>
<td>Caspofungin injection-3</td>
<td>3.167</td>
<td>8454375</td>
</tr>
<tr>
<td>Caspofungin injection-4</td>
<td>3.302</td>
<td>8470229</td>
</tr>
<tr>
<td>Caspofungin injection-5</td>
<td>3.163</td>
<td>8483608</td>
</tr>
<tr>
<td>Caspofungin injection-6</td>
<td>3.140</td>
<td>8402989</td>
</tr>
<tr>
<td>Mean</td>
<td>3.199</td>
<td>8465952</td>
</tr>
<tr>
<td>% RSD.</td>
<td>2.058</td>
<td>0.415</td>
</tr>
<tr>
<td>Std. Devitio</td>
<td>0.066</td>
<td>35157</td>
</tr>
</tbody>
</table>

Y=1651731.671x+296419.5807
r² = 0.9999
**Accuracy:** Percentage recovery of the active ingredient using this method was determined using Casporan® 50 mg is a white to off-white powder/cake for infusion in a vial with a red aluminum band and a plastic cap. Casporan® is a sterile, lyophilized product for intravenous (IV) infusion that contains a semisynthetic lipopeptide (echinocandin) compound. The results of accuracy studies from standard solution and excipient matrix were shown in Table 5; recovery values demonstrated that the method was accurate within the desired range.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Recovery at 80% dilution Level Peak areas</th>
<th>Recovery at 100% dilution Level Peak areas</th>
<th>Recovery at 120% dilution Level Peak areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6842022 7741718 8436297 9293221 10217467 11310318</td>
<td>6842022 7741718 8436297 9293221 10217467 11310318</td>
<td>6842022 7741718 8436297 9293221 10217467 11310318</td>
</tr>
<tr>
<td>2</td>
<td>6978144 7774423 8438538 9349224 10116313 11347869</td>
<td>6978144 7774423 8438538 9349224 10116313 11347869</td>
<td>6978144 7774423 8438538 9349224 10116313 11347869</td>
</tr>
<tr>
<td>3</td>
<td>6864145 7763034 8438538 9349224 10116313 11347869</td>
<td>6864145 7763034 8438538 9349224 10116313 11347869</td>
<td>6864145 7763034 8438538 9349224 10116313 11347869</td>
</tr>
<tr>
<td>Avg</td>
<td>6894770.333 7759725.0 8436562 9343570.3 10227474.33 11336654</td>
<td>6894770.333 7759725.0 8436562 9343570.3 10227474.33 11336654</td>
<td>6894770.333 7759725.0 8436562 9343570.3 10227474.33 11336654</td>
</tr>
<tr>
<td>Std.Dev</td>
<td>73046.10 16601.70 1857.73 47774.06 116487.84 22890.78</td>
<td>73046.10 16601.70 1857.73 47774.06 116487.84 22890.78</td>
<td>73046.10 16601.70 1857.73 47774.06 116487.84 22890.78</td>
</tr>
<tr>
<td>%RSD</td>
<td>1.059 0.214 0.022 0.511 1.139 0.202</td>
<td>1.059 0.214 0.022 0.511 1.139 0.202</td>
<td>1.059 0.214 0.022 0.511 1.139 0.202</td>
</tr>
<tr>
<td>% Recovery</td>
<td>98.90 98.28 106.36</td>
<td>98.90 98.28 106.36</td>
<td>98.90 98.28 106.36</td>
</tr>
</tbody>
</table>

**Sensitivity:** Limit of detection (LOD) for Caspofungin was 0.001µg/mL and limit of quantification (LOQ) for Caspofungin was 0.003µg/mL. The results of LOD and LOQ were indicating a high sensitivity of the method.

**Robustness:** The HPLC parameters were deliberately varied from normal procedural conditions including the mobile phase flow rate was 1.0 mL/min. This was changed by ±0.2 units to 0.8 and 1.2 mL/min. The effect of stationary phase was studied by the use of LC columns from different batches at 35°C. Under these variations, all analytes were adequately resolved and elution orders remained unchanged. The testing solution maintained a signal-to-noise ratio over 10 in all varied conditions. The peak resolution was all larger than 1.5 under each variation.
Table-5: Robustness study of Casporan® lyophilized product for intravenous (IV) infusion solution at 100% level (5 μg/mL):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caspofungin in Flow increase study</th>
<th>Caspofungin in Flow decrease study</th>
<th>Caspofungin in Variable column Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run time</td>
<td>Peak Area</td>
<td>Run time</td>
</tr>
<tr>
<td>Injection-1</td>
<td>2.868</td>
<td>8444635</td>
<td>3.601</td>
</tr>
<tr>
<td>Injection-2</td>
<td>2.871</td>
<td>8438791</td>
<td>3.688</td>
</tr>
<tr>
<td>Injection-3</td>
<td>2.963</td>
<td>8478020</td>
<td>3.664</td>
</tr>
<tr>
<td>Mean</td>
<td>2.901</td>
<td>8453815.3</td>
<td>3.651</td>
</tr>
<tr>
<td>% RSD</td>
<td>1.854</td>
<td>21164.53</td>
<td>1.234</td>
</tr>
<tr>
<td>Std. Dev</td>
<td>0.054</td>
<td>0.250</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Analysis of the fixed dose combination tablet:

Casporan® 50 mg is a white to off-white powder/cake for infusion in a vial with a red aluminum band and a plastic cap. Casporan® is a sterile, lyophilized product for intravenous (IV) infusion that contains a semisynthetic lipopeptide (echinocandin) compound synthesized from a fermentation product of Glarea lozoyensis. Casporan® 50 mg also contains: 39 mg sucrose, 26 mg mannitol, glacial acetic acid, and sodium hydroxide. The contents of 5 vials are emptied and their average weight was calculated and they were then finely blended. An amount of the homogenous powder equivalent to 5 mg was transferred into a 100ml volumetric flask, added 40 ml of diluents (Acetonitrile and water in the ratio of 50:50 v/v), sonicated for 30 min, diluted to 100 ml with diluents. 50ml sample taken from this solution was centrifuged at 3000 rpm for 15 min. A 5-ml aliquot from supernatant was then decanted to another 50-ml volumetric flask. Test solutions were then made up to volume with the diluent. The amount of caspofungin in standard mixtures or dosage forms were individually calculated using the related linear regression equations.

On the basis of above results, the proposed method was applied to the determination of antifungal agent capsol fungin present in freeze dried product for IV infusion. Figure-3 shows representative chromatograms obtained from the analysis of Casporan® is a sterile lyophilized product for intravenous (IV) infusion. The differences between the amount claimed and those assayed were very low and the R.S.D. values were within the acceptable range mentioned by pharmacopoeias. The mean percentage recoveries obtained after six repeated experiments were found between 97.53 and 100.98 (Table 6), indicating that the results are accurate and precise and there is no interference from the common excipients used in the pharmaceutical dosage forms.

Table-6: Assay results of Capsofungin in lyophilized product for intravenous (IV) infusion

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Label Claim (mg/powder)</th>
<th>Amount found in (mg/powder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
<td>50 mg</td>
<td>49.50 mg</td>
</tr>
</tbody>
</table>
CONCLUSION:

In this study, a validated simple and reliable RP-HPLC-PDA procedure was described for the assay of a CANCIDAS® is a sterile, lyophilized product for intravenous (IV) infusion that contains a semisynthetic lipopeptide (echinocandin) for IV infusion, which is indicated as empirical therapy for presumed fungal infections in febrile, neutropenic adult and pediatric patients. To our present knowledge, no attempts have yet been made to estimate this freeze dried product by analytical procedure. The active pharmaceutical ingredient was successfully resolved and quantified using X-Terra RP-18 Octadecyl column (250×4.6mm, 5μm) in a relatively short run time of 18 minutes in isocratic mode's chromatographic method. The proposed method provides a good resolution between active ingredients. The developed method reported herein was validated by parameters as described in ICH-Q2B guideline. System suitability, specificity, linearity, LOD, LOQ values, within- and between-day precision and accuracy of the proposed technique were obtained during the validation studies. The proposed method has the advantages of simplicity, repeatability, sensitivity and requires less expensive reagents.

REFERENCES:


