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Research Article

Development and Validation of Stability Indicating Method for the Determination of Caspofungin in Caspofungin acetate for injection Using RP-HPLC

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ABSTRACT

An isocratic reverse phase High Performance Liquid Chromatographic (RP-HPLC) stability indicating method was developed and validated for determination of Assay of Caspofungin in Caspofungin acetate for injection. The successful determination of Caspofungin was achieved using YMC-Pack Polyamine II (150×4.6 mm i.d., 5µ particle size) column maintained at 30°C temperature with mobile phase consisting 0.02 M phosphoric acid buffer of pH 3.5, acetonitrile and 2-propanol in an isocratic method. The mobile phase flow rate was 1.0 mL/min and the detection wavelength was 210 nm. The developed RP-HPLC method was validated according to ICH guidelines with respect to linearity, accuracy, precision, specificity and robustness. Validation studies demonstrated that the proposed method is simple, specific, rapid, reliable and reproducible, and this method can be applied for the routine quality control analysis of Caspofungin in Parenteral dosage form.

1. Introduction

Caspofungin Acetate is a semi synthetic lipopeptide antifungal drug, first of a new class termed 'the Echinocandins'[1,2] used in the management of Invasive aspergillosis, candidiasis (esophageal and oropharyngeal), candidemia, and other candida and aspergillus infections[3,4]. It works by inhibiting the enzyme (1 → 3)- β -D-glucan synthase and thereby disturbing the integrity of the fungal cell wall. Caspofungin was the first inhibitor of fungal (1→3)- β -D-glucan synthesis[4] to be approved by the United States Food and Drug Administration.

The chemical structure of Caspofungin Acetate is shown in figure 1, the molecular weight of the compound is 1213.42 gm/mol and the empirical formula is $C_{52}H_{88}N_{10}O_{15}.2C_2H_4O_2$. Caspofungin acetate for injection is a sterile, lyophilized product for intravenous (IV) infusion and was originally approved by both the Food and Drug Administration (FDA), in the U.S., and the EMEA, in Europe, in 2001. Few HPLC methods have been reported in the literature for quantification of Caspofungin in plasma and other biological samples[5-7]. To the best of our knowledge no stability indicating HPLC method has been reported for the estimation of Caspofungin in Parenteral dosage form. The aim of this work was to develop a rapid, simple, precise, accurate and validated HPLC method for determination of Caspofungin.

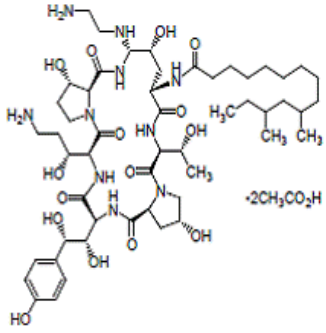

Figure 1: Chemical Structure of Caspofungin Acetate

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2. Materials and Methods

The work was carried out at the Analytical R&D department, Gland Pharma, Hyderabad. The R&D Centre is equipped with all modern, sophisticated facilities required for research, development and analysis of pharmaceuticals. Samples of Caspofungin were collected from formulation research and development department of Gland Pharma. Other chemicals procured were analytical grade.

Instruments/Equipment: Instruments used during development and validation were timely calibrated and are listed in **Table-1.**

Table 1: Instruments and Equipment used during method development and validation

S. No.	Name	Make
1.	Precision and Analytical Balance	Sartorius
2.	pH Meter	Metrohm
3.	Ultra Sonicator	SV Scientific
4.	HPLC Detector: UV-Visible / PDA	Shimadzu
5.	Water Purifier	Millipore

Chemicals/Reagents: All chemicals, reagents used were of appropriate grades for HPLC analysis and are listed in **Table 2.**

Table 2: Chemicals used during method development and validation

S. No.	Name of Chemical/Reagent	Make/Grade
1.	Phosphoric acid (85%)	Merck /GR
2.	Ammonia	Merck /GR
3.	Methanol	Spectrochem /HPLC
4.	Acetonitrile	Merck /HPLC
5.	2-Propanol	Merck /HPLC
6.	Water	MilliQ

Method: The method for determination of Caspofungin in Caspofungin acetate for injection was optimised based on different trials taken at different chromatographic conditions.

Chromatographic Conditions: The optimised chromatographic conditions are as follows

Column	:	YMC-Pack Polyamine II
Column dimensions	:	150mm x 4.6mm x 5µ
Flow Rate	:	1.0 mL/min
Injection Volume	:	20 µl
Column Oven Temperature	:	30°C
Sample Cooler Temperature	:	25°C
Detection Wavelength	:	210 nm
Run Time	:	30 minutes

Mobile Phase: 0.02 M phosphoric acid buffer, pH 3.5 was adjusted with Ammonia solution in water, Acetonitrile and 2-propanol in the ratio 28:58:14.

Diluent: Phosphoric acid buffer: Methanol 20:80 v/v

Working standard solution: 1000 µg/mL solution of Caspofungin standard.

Sample preparation for Caspofungin for injection: The conventional vial which contains 54.6mg Caspofungin (as acetate) for injection was bought to room temperature (usually stored at 2-8°C) and reconstituted by adding 11mL of 0.9% Sodium Chloride and mixed gently until a clear solution is obtained. Further diluted 10mL of above solution to 50mL with diluent. Concentration of Caspofungin is about 1000µg/mL.

3. Experimental

Method Validation: After method development, validation of the chromatographic method for Caspofungin was performed in accordance with ICH guidelines[8,11.]

Linearity and Range: Linearity was evaluated by plotting graph with concentrations (%) versus peak area of Caspofungin. A series of solutions of Caspofungin standard was prepared in the concentrations ranging from about 50% to 150% of target concentration (Standard concentration) and analyzed. The graph was plotted with individual concentrations on X-axis versus respective area on Y-axis and determined the correlation coefficient. Caspofungin standard stock solution of 2000 µg/mL was used for preparation of subsequent aliquots; aliquots of 1.5, 1.4, 1.3, 1.2, 1.1, 1.0 0.9, 0.8, 0.7, 0.6 and 0.5 mg/mL concentrations were prepared by serial dilution. Co-relation coefficient should be not less than 0.999

Accuracy: Accuracy was performed in five different levels for Caspofungin at 50%, 75%, 100%, 125% and 150% by mixing API and Placebo. Analysed samples in triplicate for each level and % Recovery was calculated. Average % Recovery at each spike level shall be not less than 97.0 and not more than 103.0. % RSD for the triplicate observations shall be not more than 2.0. Overall % RSD for the % Recovery shall be not more than 2.0.

Precision:

System Precision: The system precision was checked by using Caspofungin standard to ensure that the analytical system is precise. The retention time and area of six replicate injections of standard was measured and RSD was calculated. % RSD of the area and RT for six determinations shall not be more than 2.0.

Method Precision: Precision of the test method was determined by analysing the homogenous sample preparations by using Caspofungin formulation of a single batch for six times. The % RSD of the assay values from six determinations shall not be more than 2.0.

Specificity: Blank (Diluent), Placebo and known impurities solutions were prepared and injected to check the interference at the retention time of the caspofungin peak ands evaluated. Blank, Placebo and known impurities peaks should not show any interference at the retention time of Caspofungin peak.

Robustness: Robustness was done by changing the column temperature (±5°C), flow rate (±10%), pH of buffer solution (±0.2 units), Organic composition of mobile phase (±5%). All the system suitability parameters shall be met as per the method and the % assay difference from control condition should be not more than 1.0

Solution stability: The standard and test solution was prepared and stored at room temperature and analysed at regular time intervals for 48 hours. The % difference with respect to initial shall not be more than 2.0.

Forced Degradation Studies: Forced degradation studies were carried out on the sample preparations of Caspofungin Acetate for injection and the degradation was evaluated by calculating the % degradation of Caspofungin in comparison with unstressed sample preparation. The degradation between 10 % and 30% was tried by following stress conditions to prove that the method is has stability indicating characteristics.

Acid Stress degradation: Test preparation was subjected to acid stress degradation by treating the sample with 0.1N Hydrochloric acid solution. Alkali stress degradation: Test preparation was subjected to alkali stress degradation by treating the sample with 0.1N Sodium hydroxide solution.

Peroxide Stress degradation: Test preparation was subjected to peroxide stress degradation by treating the sample with 30% H2O2 solution

Thermal stress degradation: Test preparation was subjected to thermal treatment (80°C) for enough time. The % degradation in all stress conditions were evaluated by calculating the % assay and by comparing the assay results with the assay of unstressed sample. Minimum 10 to 30% degradation shall be achieved. The peak purity for caspofungin peak shall pass.

4. Results and Discussion

Optimization of Chromatographic conditions: The objective of the proposed work was to develop a stability indicating method for the determination of caspofungin in pharmaceutical dosage form by RP-HPLC and to validate the developed method according to USP and ICH guidelines. Several trials were carried out for accurate and precise method development. After using several columns and buffers, suitable column chemistry and good peak shape were obtained with the chromatographic conditions as mentioned above. Forced degradation studies were conducted to demonstrate that the method was stability indicating.

System Suitability: The standard chromatograms were taken for the proposed method and various system suitable parameters were recorded. The system suitability results were given in **Table 3.**

Table 3: Results of System Suitability parameters

S. No	System Suitability Parameter	Observations	Proposed Acceptance Criteria
1.	% Relative Standard Deviation for five replicate injections of Caspofungin peak in standard solution	0.2	Should be not more than 2.0
2.	Tailing factor for analyte peak in standard solution	1.0	Should be not more than 2.0
3.	The number of theoretical plates for the caspofungin peak	5095	Should be not less than 2500

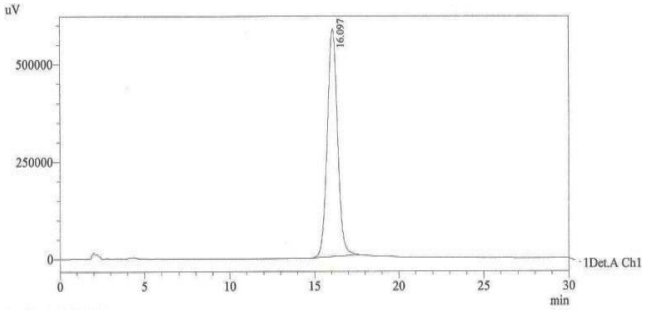

Figure 2: Chromatograms for the standard solution of Caspofungin

Linearity: The method for Caspofungin was found to be linear in the concentration range of 0.5 mg/mL to 1.5 mg/mL. Correlation Coefficient for Caspofungin is 0.999. It was observed from the data tabulated above that the system suitability parameter met the requirement of method validation (**Table 4 & Figure 3)**.

System & Method Precision: It was observed from the data tabulated in Table 5, that the retention time and area responses are consistent as evidenced by the values of relative standard deviation. Hence, it can be concluded that the system precision parameter meets the requirement of method validation. From the above results, it was concluded that the method is precise.

Table 4: Results of linearity for Caspofungin

Linearity Level (%)	Area Inj 1	Area Inj 2	Avg Response
50	12586859	12586757	12586808
60	14904378	14903960	14904169
70	17921702	17921358	17921530
80	20152708	20151868	20152288
90	22567759	22567047	22567403
100	25174177	25173053	25173615
110	27261090	27260100	27260595
120	29632556	29631814	29632185
130	32727262	32724138	32725700
140	35244120	35242000	35243060
150	37760422	37761950	37758894
Correlation coefficient (R^2)			0.999
Slope (m)			25026
Intercept (y)			57781

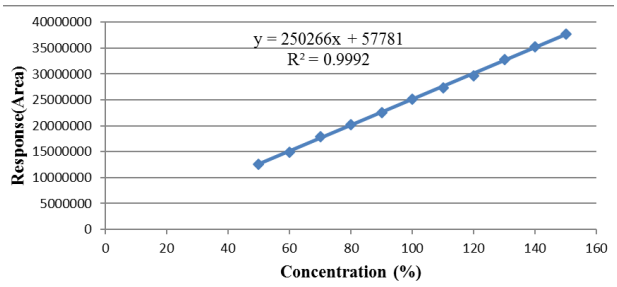

Figure 3: Linearity plot of Caspofungin

Table 5: Results of system and Method precision

System precision			Method Precision	
Injection	RT (min)	Area	Preparation	% Assay of Caspofungin
1.	16.099	24879626	1.	97.60
2.	16.096	24926310	2.	98.04
3.	16.096	24825484	3.	98.41
4.	16.097	24898472	4.	98.02
5.	16.099	24901630	5.	98.33
6.	16.102	24825491	6.	98.36
Mean	16.098	24876169		98.13
% RSD	0.01	0.17		0.3

Accuracy: From the above results, it can be concluded that the recovery is well within the limit shown in **Table 6**. Hence, the method is accurate.

Blank & Placebo Interference: Based on these chromatograms, we can say that there is no interference of blank, placebo and known impurities interference (**Table 7** and **Figure 4 & 5**).

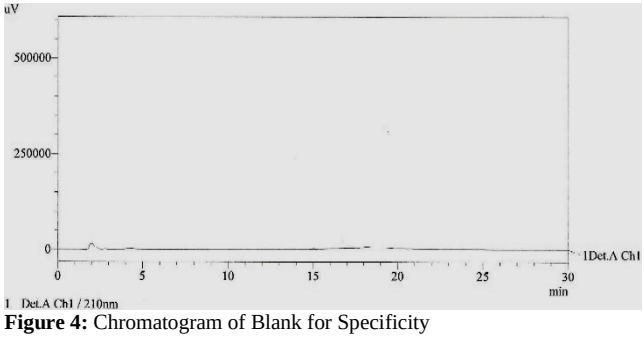

Figure 4: Chromatogram of Blank for Specificity

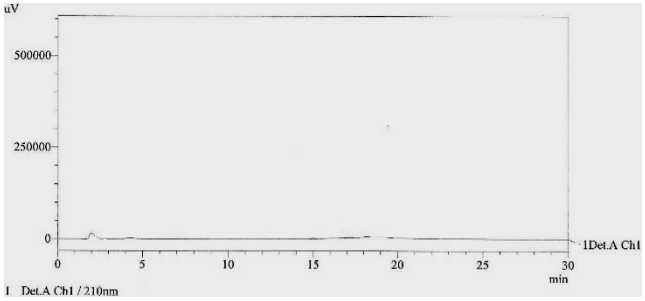

Figure 5: Chromatogram of placebo for specificity

Table 6: Results for accuracy of Caspofungin

Recovery/ Spike Level at about (in %)	Amount added (mg/mL)	Amount found (mg/mL)	% Recovery	Avg	%RSD
50	0.496	0.499	100.60		
50	0.496	0.499	100.60	100.54	0.12
50	0.496	0.498	100.40		
75	0.745	0.744	99.87		
75	0.745	0.744	99.87	99.91	0.08
75	0.745	0.745	100.00		
100	0.993	0.988	99.50		
100	0.993	0.984	99.09		
100	0.993	0.986	99.30	99.30	0.17
100	0.993	0.984	99.09		
100	0.993	0.987	99.40		
100	0.993	0.987	99.40		
125	1.241	1.228	98.95		
125	1.241	1.214	97.82	98.20	0.66
125	1.241	1.214	97.82		
150	1.489	1.450	97.38		
150	1.489	1.470	98.72	97.78	0.84
150	1.489	1.448	97.25		

Table 7: Retention times for various impurities of Caspofungin and Caspogungin

S.No	Impurity/Analyte name	Retention time
1	Impurity A	14.3
2	Caspofungin	16.1
3	Caspofungin C_o	19.1
4	Impurity B_1+B_2	21.7
5	Pneumocandine B_o	24.3

Robustness: From the **Table 8** data, all the system suitability parameters of methods is complying, and it is concluded that the method is robust.

Table 8: Results of Robustness

Parameter	Condition	The % assay difference from control condition
pH of Buffer solution by ±0.2 Units	pH 3.3	0.63
	pH 3.7	0.41
Organic composition of Mobile phase by ±5%	28:55:13*	0.84
	33:61:15*	0.61
Flow rate by ±10%	0.9mL/min	0.47
	1.1mL/min	0.85
Column Oven Temperature by ±5ºC	25ºC	0.39
	35ºC	1.2

*Buffer:ACN:IPA

Forced degradation studies: From the **Table 9 & 10** observed that the proposed acceptance criteria meet the requirements for acid and alkali degradation, and it is stable even when more stress conditions like peroxide and thermal stress is applied. Based on the forced degradation studies, the proposed analytical method can be considered as stability indicating method and can be used for release and stability studies for effective evaluations.

Table 9: Results of solution stability

Solution Stability of Std. preparation			Solution Stability of test preparation	
Time (hrs)	Area Response	% difference	Area Response	% difference
0	24862276	NA	24833852	**NA**
4	24890588	0.3	24738129	0.4
8	24851005	0.1	24802008	0.1
12	24834946	0.1	24683043	0.6
20	24854767	0.4	24816250	0.1
24	24784717	0.7	24804500	0.1
28	24755959	0.3	24726161	0.4
32	24736987	0.1	24631274	0.8
40	24737183	0.5	24773519	0.2
48	24703338	0.2	24695811	0.6

Table 10: Results of Forced degradation studies

Condition	% degradation achieved	Purity Angle	Purity Threshold	Pass/ Fail
Acid	25.17	0.035	0.237	Pass
Alkali	22.46	0.032	0.244	Pass
Peroxide	26.5	0.030	0.239	Pass
Heat	17.21	0.032	0.244	Pass

5. Conclusion

A novel, reverse phase liquid chromatographic method has been developed and validated for the estimation of Caspofungin in Caspofungin Acetate for Injection. Validation revealed that the method is specific, accurate, precise, reliable and reproducible. Calibration plots were linear over the concentration ranges 0.50-1.50 mg/mL for Caspofungin. Recovery was in the range 97.78–100.54% for Caspofungin and the coefficient of variance was <2.0%. The high percentage recovery and low co-efficient of variation confirm the suitability of the method for analysis of Caspofungin in Pharmaceutical dosage form. Hence, it can be successfully used for the routine analysis of Caspofungin in pharmaceutical dosage form.

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Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

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