Chemistry Research Journal, 2023, 8(6):85-100
Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Method Development and Validation of Caspofungin and Voriconazole combination by RP-HPLC Method

Anita Bundela*, Sachin K Jain, Sudha Vengurlekar

*Oriental College of Pharmacy & Research, Oriental University, Near Aurobindo Hospital Sanwer Road Indore 453555
*Corresponding author E-mail: anita14bundela@gmail.com

Abstract
The main objective of this study was to develop and validate a robust High-Performance Liquid Chromatography (HPLC) method for the simultaneous quantification of voriconazole and caspofungin in both Active Pharmaceutical Ingredients (API) and injectable formulations, adhering to the guidelines set by the International Conference on Harmonisation (ICH). The validation process encompassed a linear concentration range of 10-50 µg/ml, yielding high correlation coefficients of 0.9993 for voriconazole and 0.9997 for caspofungin acetate. Precision was established, with the % RSD of the peak response from three replicate injections of standard concentrations being below 2 %, indicating the method's precision. Accuracy was assessed by calculating the percentage recoveries of the API from dosage forms, ranging from 99.70% to 100.40% for voriconazole and 99.12% to 99.55% for caspofungin acetate, demonstrating accuracy. The method exhibited high sensitivity with low limits of detection (LOD) of 0.003 µg/ml for voriconazole and 0.09 µg/ml for caspofungin acetate. In conclusion, this proposed RP-HPLC method is straightforward, sensitive, reliable, and can be applied for the simultaneous determination of voriconazole and caspofungin acetate in bulk samples and pharmaceutical formulations as required for specific analytical needs and situations.

Keywords: High-Performance Liquid Chromatography, Pharmaceutical Ingredients, Voriconazole, Caspofungin, RP-HPLC

1. Introduction

In modern analytical laboratories, the utilization of advanced analytical tools and techniques has become standard practice. The continuous advancements in instrument automation have greatly broadened our understanding of various fields. Moreover, traditional manual procedures in the realm of analytical investigations have progressively given way to instrumental approaches [1-5]. Analytical techniques generally fall into two major categories:

Qualitative Analysis: This type of analysis is focused on identifying the components or constituents present in a given sample. It aims to determine what substances are present, without quantifying their amounts.

Quantitative Analysis: Quantitative analysis is concerned with determining the exact or relative amounts of specific substances within a sample. It provides precise measurements of the quantity of a particular species or compound in a sample.

Structural Analysis: Structural analysis is a specialized form of chemical analysis that aids in elucidating the spatial arrangement of atoms in a molecule. It can also help identify the presence or position of specific organic functional groups within a compound. The mentioned Table 1 likely provides an overview of various instrumental methods used

in the pharmaceutical field and their primary applications. These methods are essential for conducting both qualitative and quantitative analyses, as well as structural analysis, to ensure the quality, safety, and efficacy of pharmaceutical products.

Table 1: Various Instrumental Methods of Analysis [6]

Instrumental Method	Property Measured	Application
UV- Visible Spectrophotometry	Absorption of radiation	Identification of the functional group, Quantitation of unsaturated compounds.
IR Spectroscopy	Absorption of radiation	Identification of the functional groups.
Atomic Absorption Spectroscopy	Absorption of radiation	Quantitation of metals or metalloids.
Flame Photometry	Emission of radiation	Quantitation of alkali metals or alkaline earth metals.
X-Ray Diffraction	Diffraction of radiation	Identification of crystal lattice structure. Determination of percent of crystallinity in polymers.
Nuclear Magnetic Resonance (NMR) • LC-NMR	Nuclear spin energy level of a mol in an applied magnetic field.	Identifies type of hydrogen and carbon in organic molecules.
Thermal analysis • (DTA \ DSC)	Difference in Temperature or heat energy	Determination of melting point, Polymorphism, Drug – excipients compatibility
Mass spectrometry • LC-MS \ GC-Ms	Mass to charge ratio (m\e)	Mol. Wt. determination • Quantification of the analyte in liquid or gas sample. • Analysis of the biological sample. • Analysis of trace impurity and degradants.

Pharmaceutical Analysis and Need of Drug Analysis

Pharmaceutical analysis plays a pivotal role in pharmaceutical chemistry, encompassing the science of extracting, preparing, purifying, and estimating chemical substances used in the formulation of medicines and drug products. It is a cornerstone of the pharmaceutical industry, contributing significantly to its evolution and functioning. Analytical research and development represent a process-oriented function that guarantees the identity, safety, efficacy, purity, and quality control of pharmaceutical products [7].

The pharmaceutical market witnesses a continuous influx of new drugs each year, including entirely new compounds or modified versions of existing ones. There is often a time gap between a drug's introduction to the market and its inclusion in pharmacopoeias. This lag is due to various factors, including uncertainties surrounding the drug's extended and broader usage, reports of new toxicities (which might lead to its removal from the market), the development of patient resistance, and the introduction of superior drugs by competitors. In such circumstances, established standards and analytical procedures for these drugs may not be available in pharmacopoeias. Consequently, it becomes imperative to develop new analytical methods to assess and ensure the quality and safety of these pharmaceutical products.

2. Materials and Instruments

Chemicals and solvents

- Acetonitrile - HPLC grade (Qualigens)
- Methanol - Spectrochem /HPLC
- Water (Milli Q) - In house

Chromatogarphic Conditions and Analytical Method Development

The simultaneous estimation of drugs was conducted using an isocratic high-performance liquid chromatography (HPLC) system. The HPLC system consisted of two LC-20AD pumps manufactured by Shimadzu® Japan, a programmable SPD-M20A photodiode array detector also manufactured by Shimadzu® Japan, a CBM-20A system controller, and LC solution software provided by Shimadzu® Japan. The detection of drugs was performed using Hypersil TM Thermo Scientific "ODS-C18 (250 mm × 4.6 mm, i.d.2.5 µm)" columns, with a detection wavelength set at 215 nm. The experimental duration of the sample was determined to be 15 minutes, with an injection volume of 20 µl. The flow rate of the isocratic mobile phase was determined to be 1 ml/min. The mobile phase consisted of Milli-Q water and acetonitrile in a 1:1 ratio. A membrane filter with a porosity of 0.45 µm was used to filter the mobile phase, which was then degassed for further processing [8].

Wavelength Selection

The drugs were dissolved in methanol and then diluted with the mobile phase to achieve a concentration of 10 µg/ml. The resulting solution was then filtered and subjected to individual scanning using a UV-Visible spectrophotometer within the wavelength range of 200-400nm. The spectral regions were superimposed in order to get the isosbestic point. The wavelength of 215 nm has been established as a constant parameter for further investigations [9].

Preparation of Mobile Phase

The mobile phase used in this investigation consisted of Milli-Q water and acetonitrile at a volumetric ratio of 1:1. Filtration was conducted using a 0.45-micron nylon membrane filter (Millipore), and an ultrasonic bath was used for degassing prior to subsequent usage in order to prevent column obstruction and clogging caused by minute particles. The standard and sample preparations were conducted using methanol as the diluent.

Preparation of standard solutions

The standard stock solutions of voriconazole and caspofungin acetate, each with a concentration of 1.0 mg/ml, were generated by dissolving 10 mg of the respective medications in 10 ml of methanol. The mixture was then subjected to sonication for a duration of 15 minutes using a sonicator (Leela Sonic-200, India) to ensure full dissolution of the pharmaceuticals. The major standard stock solution volumes (0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ml) were diluted to a final volume of 10 ml using a diluent composed of Milli-Q water and Acetonitrile in a 1:1 % v/v ratio. For the investigation, the final concentration of both medications ranged from 10 to 100 µg/ml. The final solution underwent filtration using a 0.45 µ nylon Millipore membrane filter [10].

Sample Preparation

The Vorier Injection, containing voriconazole at a concentration of 200mg, was reconstituted using Milli-Q water to get a final volume of 20ml. A concentration of voriconazole of 10mg/ml was successfully attained. In a similar manner, the reconstitution of Cancidas Injection (caspofungin acetate-50mg) included the addition of Milli-Q water to achieve a final volume of 10 ml. A concentration of caspofungin acetate at 5mg/ml was attained. A volume of 1.0 mL of voriconazole solution and 2.0 mL of caspofungin acetate solution were extracted and afterwards put into a 100 mL volumetric flask that had been cleaned and dried. The solutions were diluted to a final volume of 100 ml using the mobile phase and then filtered using a Millipore membrane, resulting in concentrations of 100µg/ml. The concentrations underwent further processing in order to achieve a concentration of 10µg/ml for both medications. The experimental specimen underwent several injections of a 20 µl solution at a flow rate of 1 ml/min. The wavelength used for detection was optimized at 215 nm [11].

3. Validation of Analytical Method

The methodology was verified in accordance with the requirements outlined in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2 (R1). The validation parameters were established.

Specificity

The determination of method specificity included the evaluation of standard chemicals in order to identify and mitigate any interferences. The method's specificity was evaluated by comparing the chromatograms obtained from the drug sample containing the widely used excipient combination with those obtained from the blank solution. The blank solution was generated by combining the excipients in the mobile phase, except the pharmacological component. It is recommended that there be no interference from blank or placebo peaks at the Retention Time (RT) of the primary peak and known impurity peaks [12]. The minimum acceptable value for Peak Purity is 0.9.

Two vials containing lyophilized placebo for each medicine were reconstituted with water individually. The resulting solutions were then put into separate 100 mL volumetric flasks, mixed well, and afterwards brought to the desired volume using a diluent. The dilutions were generated in a manner consistent with the sample that was injected into the High-Performance Liquid Chromatography (HPLC) system, according to the specified parameters outlined in the test procedure. The method's specificity was determined using peak purity profiling investigations. Prior to injection, the mixed placebo sample underwent filtration using a 0.45µ membrane filter.

Linearity

The drug's quantitative measurement was achieved using the external standard technique. Prior to use, the mobile phase underwent filtration using a membrane filter with a pore size of 0.45µ. The flow rate of the mobile phase was set at 1 ml/min. The column was subjected to equilibration with the mobile phase for a minimum duration of 30 minutes before the drug solution was injected. The temperature of the column was consistently maintained at 25±10°C throughout the duration of the investigation [13].

The linearity of the peak area response was assessed by doing six duplicate measurements at seven different concentration points. To generate working dilutions of the sample, a series of dilutions ranging from 10-50 µg/ml were created. This was achieved by taking appropriate dilutions of the standard solutions in separate 10 ml volumetric flasks, which were then diluted with the mobile phase up to the designated mark. A volume of 20 microlitres from the dilutions was injected into the column during each iteration, with a flow rate of 1 ml/min. The column received six injections for each dilution. The concentration of medicines in the eluents was measured using a spectrophotometer at a wavelength of 215 nm, and the resulting chromatograms were acquired [14].

Accuracy

The objective of this research was to evaluate the degree of concordance between the outcomes acquired via the suggested methodology and the genuine value. The correctness of the suggested approach was verified by the performance of recovery tests using the usual addition method. A predetermined amount of a pure drug substance was introduced into a pre-analyzed sample, and the resulting mixture was subjected to reanalysis using the suggested methodology. The percentage of the drug substance that was successfully recovered was afterwards determined and reported. The percentage of recovery within the range of 50% to 200% should ideally fall between 90.0% and 110.0%, ensuring a satisfactory degree of accuracy. According to the literature [15], it is recommended that the percentage recovery at the limit of quantification (LOQ) level should fall within the range of 80.0% to 120.0%.

The accuracy of the procedure was assessed using the usual addition method. The experimental procedure included the addition of varying concentrations (50%, 100%, and 150%) of Caspofungin and Voriconazole, in their pure form, to a pre-analyzed working standard solution of the medications. The provided approach included the analysis of the sample solutions in triplicate at each stage. The percentage of individual recovery and the percentage of relative standard deviation (% RSD) for recovery at each level were computed [16].

Preparation of 50% recovery sample

A 0.5ml aliquot of standard stock solutions containing Caspofungin and Voriconazole at a concentration of 5µg/ml was combined with a 1ml aliquot of a mixed sample stock solution at a concentration of 10 µg/ml in a 10 ml volumetric flask. The volume was then adjusted with the mobile phase to reach the mark, resulting in a 50% recovery sample with a concentration of 15 µg/ml.

Preparation of 100% recovery sample

A 1 milliliter aliquot of standard stock solutions of Caspofungin and Voriconazole was combined with a 1 milliliter aliquot of a mixed sample stock solution containing 10 micrograms per milliliter. The resulting mixture was then transferred to a 10 milliliter volumetric flask and diluted with the mobile phase up to the mark, resulting in a 100% recovery sample with a concentration of 20 micrograms per milliliter.

Preparation of 150% recovery sample

A total of 1.5 milliliters of standard stock solutions of Caspofungin and Voriconazole were combined with 1 milliliter of the sample stock solution, which had a concentration of 10 micrograms per milliliter (µg/ml). This mixture was then transferred to a 10 milliliter volumetric flask and diluted with the mobile phase until the flask was filled to the mark, resulting in a 150% recovery sample with a concentration of 25 µg/ml.

The solutions underwent filtration using a 0.45µ membrane filter and were subsequently analyzed using the RP-HPLC method according to the specified chromatographic conditions. The recovery studies were conducted in triplicate for each level [17].

Precision

The objective of this study is to demonstrate the precision of the developed reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous analysis of Caspofungin and Voriconazole. Precision refers to the extent of reproducibility exhibited by an analytical method when implemented in typical operational circumstances. The study assessed the precision of the method in terms of its repeatability (intra-day assay) and intermediate precision (inter-day assay) [18]. The study of method repeatability involved conducting the assay five times within a single day to assess intra-day precision. Additionally, intermediate precision was evaluated by repeating the assay on two separate days to examine inter-day precision. The relative standard deviation (RSD) value was calculated to quantify the precision of the system.

Robustness

The robustness of an analytical method refers to its ability to withstand intentional small variations in method parameters without being affected. It serves as an indicator of the method's reliability under normal operating conditions. A research investigation was carried out to assess the impact of intentional modifications in the optimized chromatographic parameters, such as the mobile phase composition, flow rate, and column temperature. The impact of these modifications on the system suitability parameters, such as the tailing factor and number of theoretical plates, as well as on the assay, was investigated. Only one condition was changed at a time while keeping all other parameters consistent [19].

An experimental variation of ±0.2ml/min was introduced in the flow rate, and the percentage relative standard deviation was calculated using statistical methods. The method's robustness was assessed by varying the flow rate to 0.8 ml/min and 1.2 ml/min, deviating from the specified flow rate of 1 ml/min.

Limit of Detection (LOD)

The limit of detection (LOD) is a quantitative measure that represents the minimum concentration of an analyte that produces a detectable response. The limit of detection (LOD) for high-performance liquid chromatography (HPLC) methods is typically established by ensuring a signal to noise ratio (S/N) of three times (S/N ratio ~ 3). In this study, the analyte at the lowest concentration within the calibration range was measured and quantified using six replicates.

Limit of Quantification (LOQ)

The limit of quantification (LOQ) is a parameter that establishes the minimum concentration at which a substance can be accurately and precisely measured. The limit of quantification is defined as the minimum concentration at which the precision, as indicated by a relative standard deviation (RSD) of less than 2%, is achieved.

4. Method Development

The primary aim of this study was to develop and verify a HPLC technique that is straightforward, exact, dependable, quick, highly accurate, and requires minimal time. This method will be used to simultaneously analyze voriconazole and caspofungin in both the bulk and injectable formulation, in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. Based on the analysis of existing literature, it was determined that there is currently no established technique for the concurrent determination of voriconazole and caspofungin using the RP-HPLC method.

Selection of wavelength

A solution containing 10 µg/ml of voriconazole and a separate solution with 10 µg/ml of caspofungin acetate were prepared using methanol as the solvent, and the mobile phase was used as the diluent. These prepared solutions were individually scanned across the UV-Visible spectrum from 190 nm to 400 nm using a spectrophotometer. The optimal response for the overlain spectrum of voriconazole and caspofungin acetate was observed at 215 nm. As a result, the entire method was executed at a wavelength of 215 nm.

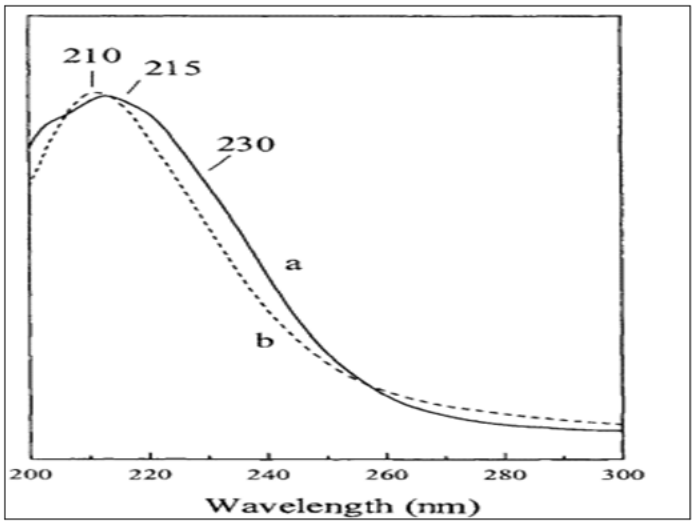

Figure 1: Overlay UV spectrum of caspofungin acetate (a) and voriconazole (b)

System suitability

The method was optimized for the simultaneous determination of voriconazole and caspofungin acetate in a pharmaceutical dosage form. System suitability testing is a critical step in validating analytical techniques and confirming the resolution between multiple peaks of interest. In this study, all essential parameters (theoretical plates, retention time, and tailing factor) consistently met the predefined acceptance criteria. The retention time for voriconazole was determined to be 3.661 minutes, while for caspofungin acetate, it was 5.116 minutes. The tailing factor was below 2, and the theoretical plate count exceeded 2000, indicating that the method performed well under the specified conditions, as presented in Table 2. The resolution between the two analytes was excellent, with no observed peak asymmetry or interference from other impurities. All results fell within the defined acceptance criteria, confirming the successful optimization of the method.

Table 2: System suitability of voriconazole and caspofungin acetate

S. No.	Name of the drug	Retention Time	Area
1.	Voriconazole	3.624	3429045
2.	Caspofungin acetate	5.178	320204
3.	Voriconazole and Caspofungin acetate standard solution	3.624 & 5.178	3429046 & 320204
4.	Voriconazole and Caspofungin acetate sample solution	3.635 & 5.174	3402667 & 318846

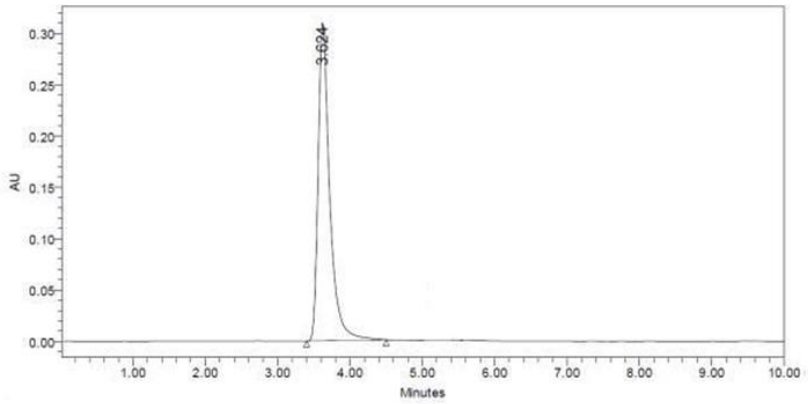

Figure 2: Standard chromatogram for voriconazole

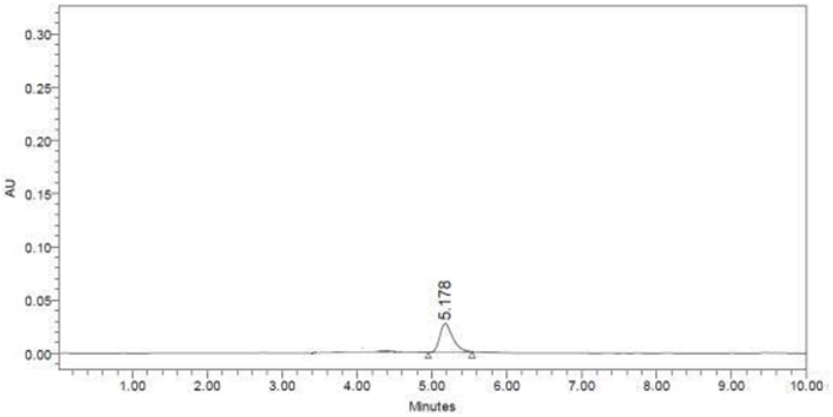

Figure 3: Standard chromatogram for caspofungin acetate

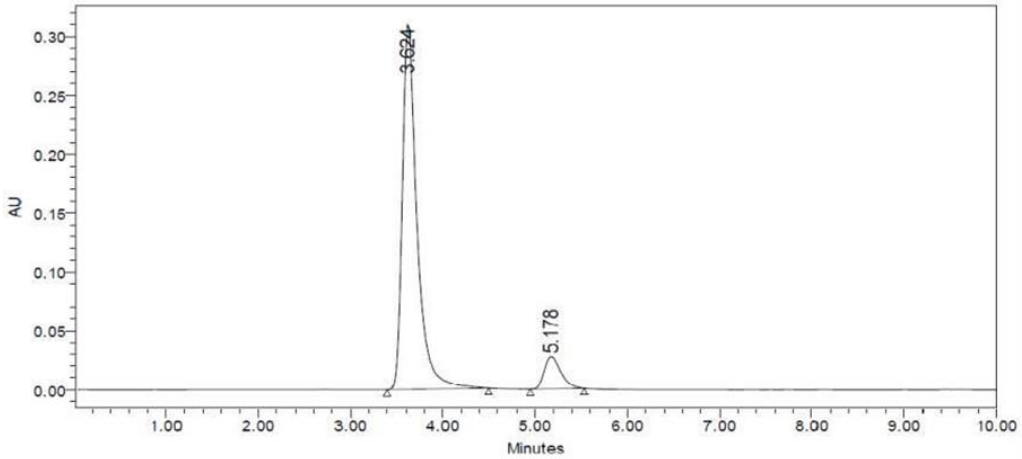

Figure 4: Standard chromatogram for optimized method

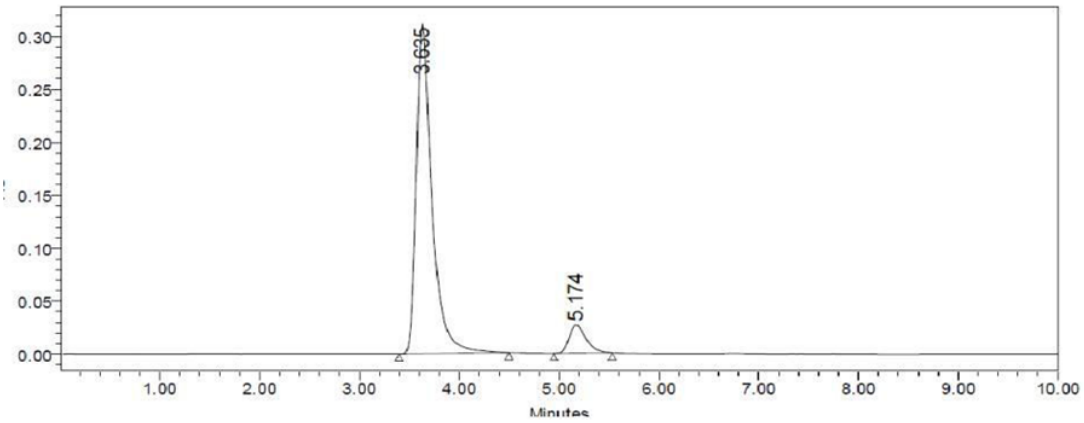

Figure 5: Chromatogram for sample solution

5. Result & Discussion

Specificity

The chromatograms of the standard and sample exhibit identical patterns with nearly identical retention times. There is no evidence of interference from the placebo or sample at the retention time of the analyte, demonstrating the specificity of the method. The chromatograms for the specificity studies, including the standard, sample, placebo, and blank, are illustrated in Figures 6 to 9.

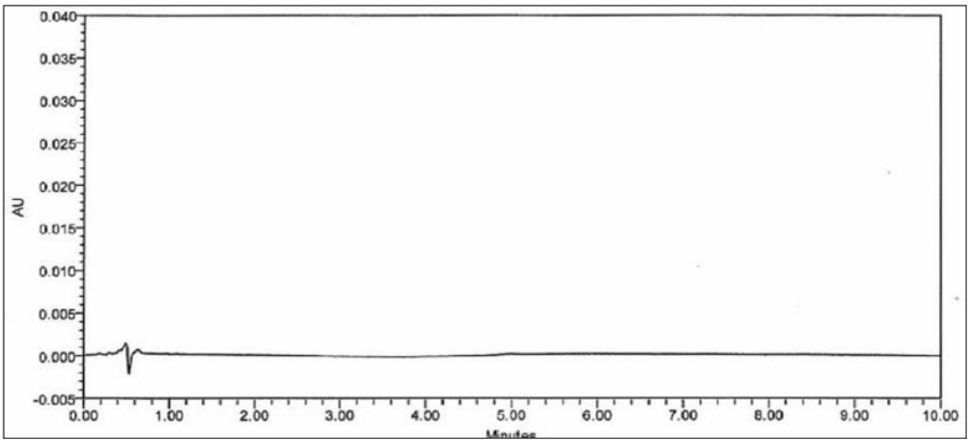

Figure 6: Chromatogram for blank interference

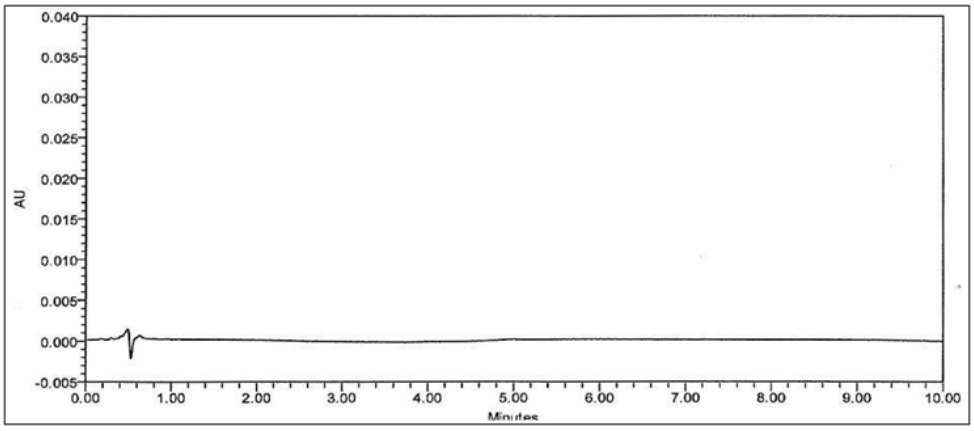

Figure 7: Chromatogram for placebo interference

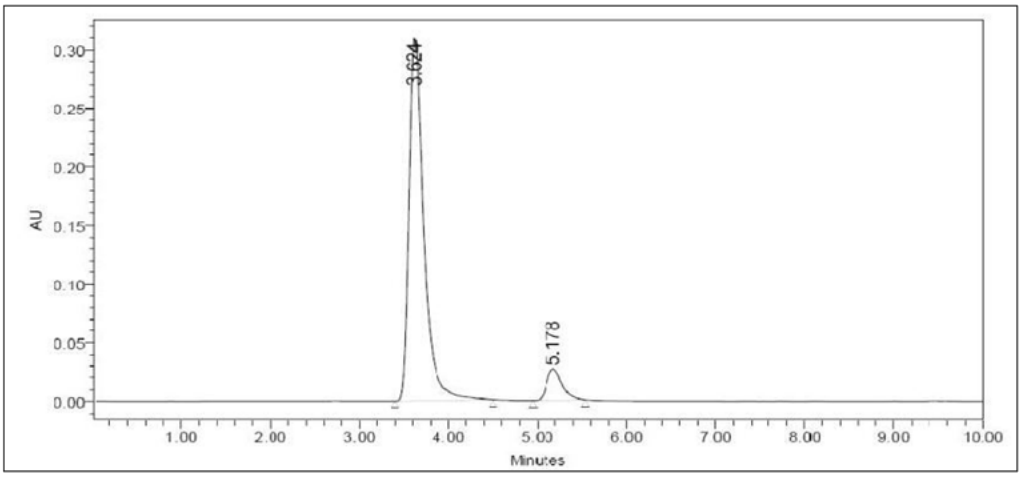

Figure 8: Standard chromatogram for Voriconazole and Caspofungin acetate

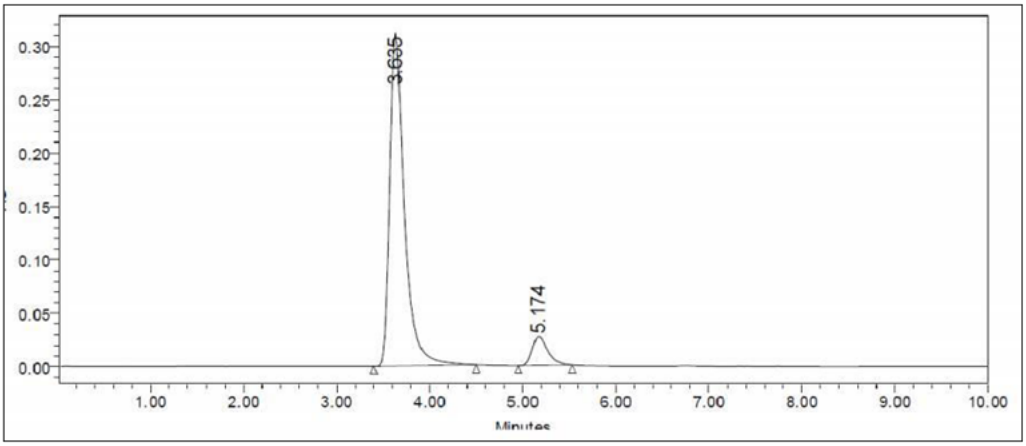

Figure 9: Sample chromatogram for Voriconazole and Caspofungin acetate

Linearity

A linearity study was conducted within a concentration range of 10-50 µg/ml. The linearity curves have been plotted and are displayed in Figures 10 and 11. The data from the linearity study is presented in Tables 3 and 4. The correlation coefficients for voriconazole and caspofungin acetate were determined to be 0.9993 and 0.9997, respectively. These results indicate a robust relationship between detector response and drug concentration levels within the specified concentration range.

Table 3: Linearity table of voriconazole

S. No.	Linearity level	Concentration (µg/ml)	Area
1.	I	10	2011514
2.	II	20	2691557
3.	III	30	3460741
4.	IV	40	4161134
5.	V	50	4964755
Correlation Coefficient			0.9993

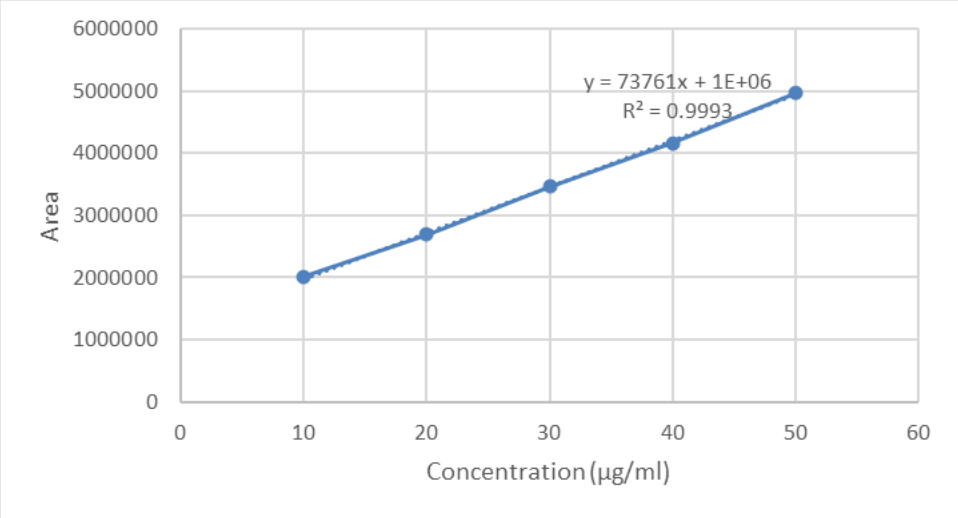

Figure 10: Calibration Curve for voriconazole

Table 4: Linearity table of caspofungin acetate

S. No.	Linearity level	Concentration (µg/ml)	Area
1.	I	10	189398
2.	II	20	258339
3.	III	30	321805
4.	IV	40	394694
5.	V	50	459759
	Correlation Coefficient		0.9997

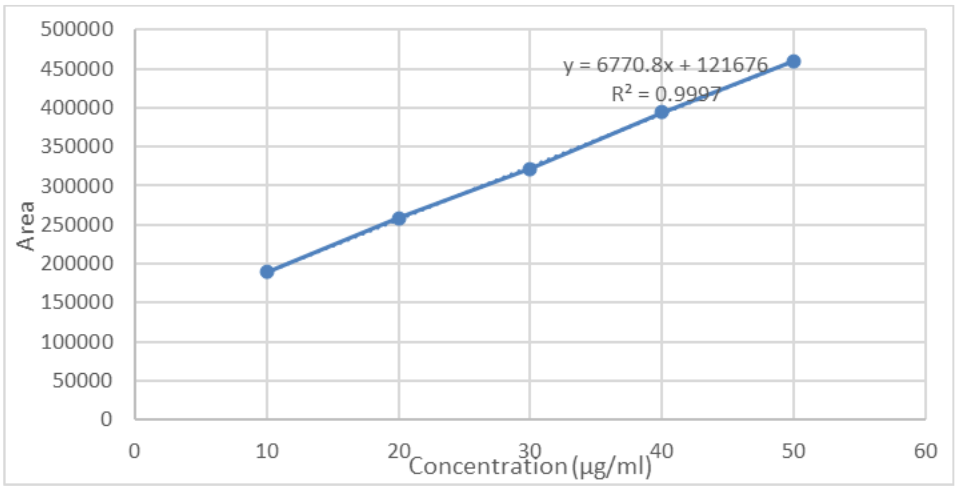

Figure 11: Calibration Curve for caspofungin acetate

Accuracy

The percentage recoveries of the pure drug from the analyzed formulation solution were calculated within a recovery range of 50% to 150%. The recovery values for voriconazole ranged from 99.70% to 100.40%, and for caspofungin acetate, they ranged from 99.12% to 99.55%. Standard and sample chromatograms for accuracy can be observed in Figures 12-14. A summary of the accuracy results is provided in Table 5.

Table 5: Accuracy table of Voriconazole and caspofungin acetate

Name of the drug	Spiked amount	Amount of drug (Standard) µg	Added drug µg	Total Drug (µg)	Total Found (µg) Mean±SD	% Recovery
Voriconazole	50%	5	10	15	14.96	99.73
	100%	10	10	20	19.94	99.70
	150%	15	10	25	25.1	100.40
caspofungin acetate	50%	5	10	15	14.92	99.46
	100%	10	10	20	19.91	99.55
	150%	15	10	25	24.78	99.12

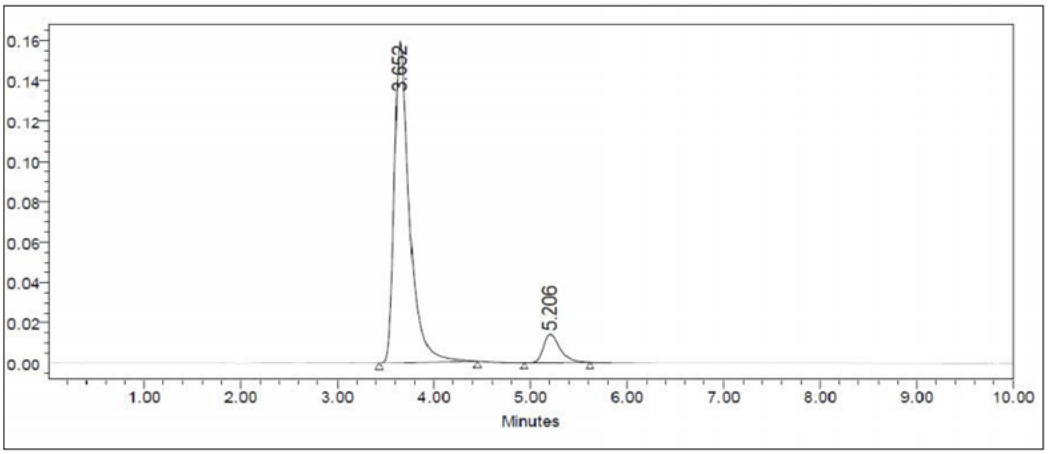

Figure 12: Chromatogram for accuracy for 50 % Conc.

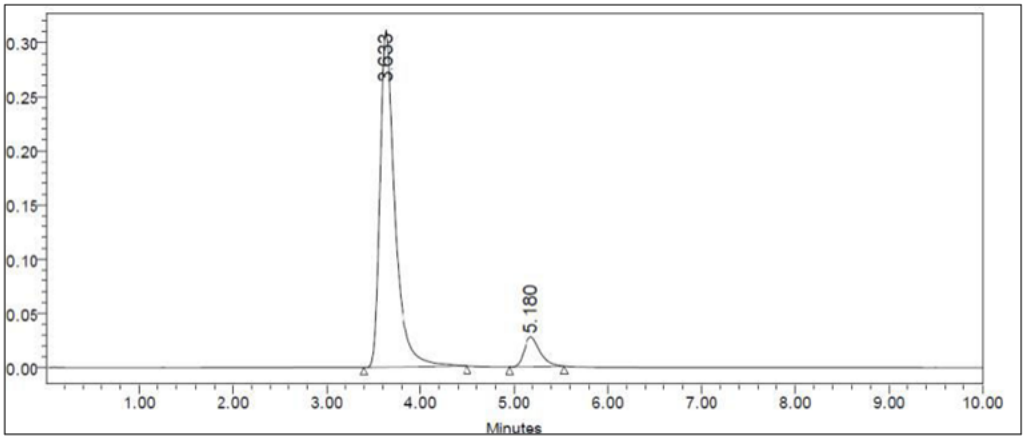

Figure 13: Chromatogram for accuracy for 100 % Conc.

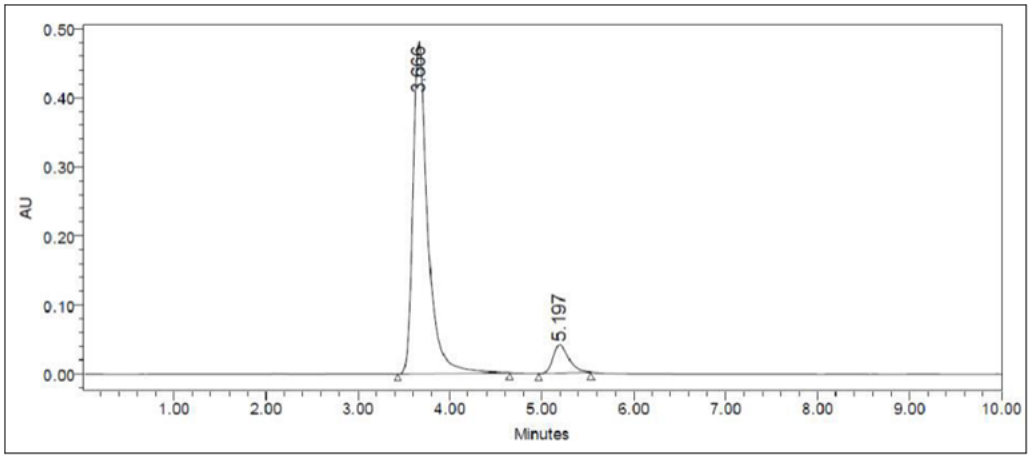

Figure 14: Chromatogram for accuracy for 150 % Conc.

Precision

The Relative Standard Deviation (%RSD) for the % Recovery of Voriconazole and Caspofungin Acetate chromatograms in terms of repeatability precision and intermediate precision was calculated. The method demonstrated satisfactory repeatability and intermediate precision. Detailed precision results are presented in Table 6.

Table 6: System and method precision of Voriconazole and Caspofungin acetate

	System		Precision	
Injection Number	**Peak areas of Voriconazole**	**Peak areas of Caspofungin acetate**	**% recovery Voriconazole**	**% recovery Caspofungin acetate**
1	3480636	323863	99.4%	99.2%
2	3463599	325248	100%	99.8%
3	3498779	322052	99.0%	99.2%
4	3497870	328133	99.8%	99.4%
5	3490276	328655	99.2%	100%
Mean	3486232	325590	99.48%	99.52
SD (±)	14601.3	2802.3	0.415	0.33
RSD (%)	**0.42**	**0.86**	**0.42**	**0.36**
Acceptance criteria	%RSD should not be morethan2			

Intermediate precision

Comparison of the results obtained on two different days demonstrates that the assay method is rugged. The results of intermediate precision (ruggedness) fall within acceptable limits and are presented in Tables 7 and 8.

Table 7: Intermediate precision results (Day-1)

Parameter	**PeakArea**	**%Assay**
Voriconazole		
Avg*	3486743	99.10%
%RSD*	0.41	0.38
Caspofungin acetate		
Avg*	3281662	99.98%
%RSD*	0.98	0.49

Table 8: Intermediate precision results (Day-2)

Parameter	PeakArea	%Assay
Voriconazole		
Avg*	3486232	99.48%
%RSD*	0.42	0.42
Caspofungin acetate		
Avg*	325590	99.52%
%RSD*	0.86	0.36

Robustness

The robustness of the analytical procedure was determined and developed by varying the flow rate, wavelength, and mobile phase composition. The robustness data for both drugs are presented in Table 9.

Table 9: Robustness of Voriconazole and Caspofungin acetate

Condition		Voriconazole			Caspofungin acetate		
		%RSD	Tailing Factor	% Recovery	%RSD	Tailing Factor	%Recovery
Change in Flow rate	Normal Flowrate (1.0ml/min)	0.78	1.09	100.18	0.87	1.11	100.67
	Change in flow rate (0.8ml/min)	0.89	1.11	98.57	0.91	1.14	100.89
	Change in flow rate (1.2ml/min)	0.78	1.02	99.45	0.78	1.32	100.56
Change in Wavelength	Normal:Wave length 215nm	0.34	1.08	99.46	0.67	1.00	100.67
	Wavelength210nm	0.56	1.1	101.78	0.78	1.15	100.45
	Wavelength220nm	0.67	1.31	98.99	0.23	1.35	100.18
Change composition of mobile phase	Normal Condition (Water: ACN) (1:1)	0.18	1.02	99.18	0.45	1.19	101.11
	(Water: ACN) (1:1.5)	0.81	1.03	99.87	1.01	1.11	100.23
	(Water: ACN) (1:2)	0.64	1.31	99.46	0.34	1.26	99.89

Limit of detection (LOD)

The limit of detection was determined using the slope and standard deviation of intercepts from the calibration curve method. The limit of detection was found to be 0.003 µg/ml for Voriconazole and 0.09 µg/ml for Caspofungin acetate. The corresponding chromatograms are depicted in Fig 15-16.

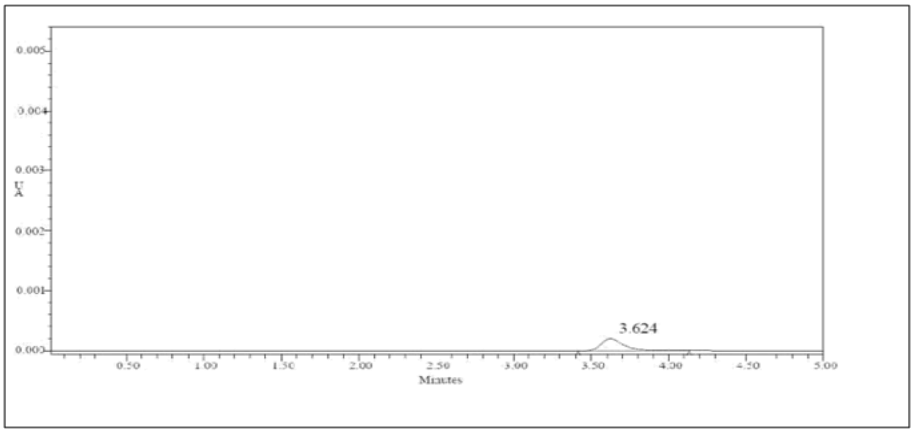

Figure. 15: LOD chromatogram of Voriconazole

Calculation of S/N ratio

The average baseline noise obtained from the blank was 52 µV, and the signal obtained from the LOD (Limit of Detection) solution, which is at 0.25% of the target assay concentration, was 154 µV. The Signal-to-Noise (S/N) ratio is calculated as follows:

S/N = Signal / Noise

S/N = 154 µV / 52 µV

S/N ≈ 2.96

The Signal-to-Noise ratio for the LOD solution is approximately 2.96.

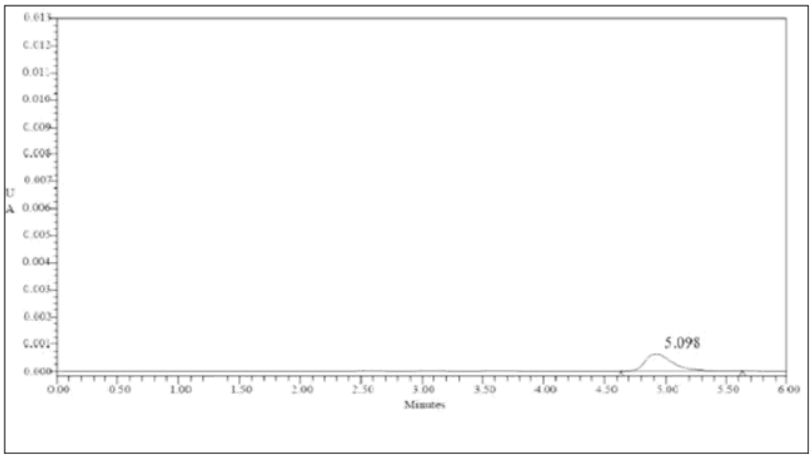

Figure 16: LOD chromatogram of Caspofungin acetate

Calculation of S/N ratio

The average baseline noise obtained from the blank was 52 µV, and the signal obtained from the Limit of Detection (LOD) solution, which is at 0.3% of the target assay concentration, was 155 µV. The Signal-to-Noise (S/N) ratio is calculated as follows:

S/N = Signal / Noise

S/N = 155 µV / 52 µV

S/N ≈ 2.98

The Limit of Detection (LOD) was found to be 2.96 for Voriconazole and 2.98 for Caspofungin acetate, which is not more than 3 as per the specified limit.

Limit of Quantification (LOQ)

The limit of quantification (LOQ) was calculated using the linearity curve method, taking into account the slope and standard deviation of intercepts from the calibration curve figure 17-18.

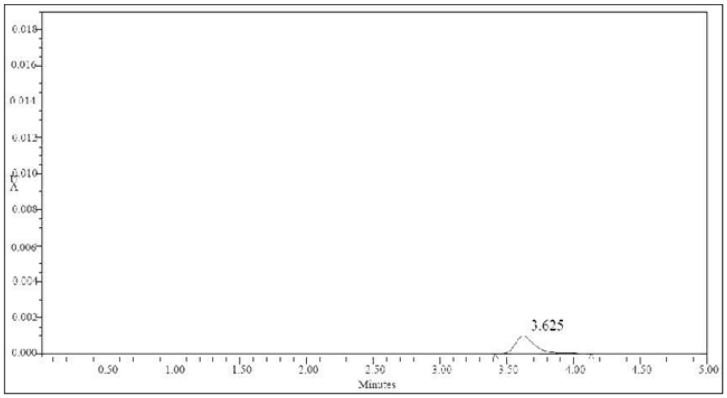

Figure 17: LOQ Chromatogram of Voriconazole

Calculation of S/N ratio

Average baseline noise obtained from blank: 52 µV

Signal obtained from LOQ solution (1% of target assay concentration) : 522µV S/N = 522/52 = 10.

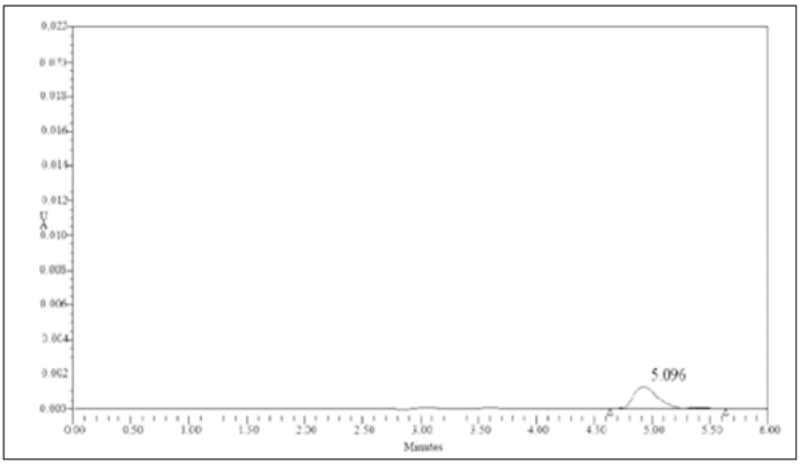

Figure 18: LOQ Chromatogram of Caspofungin acetate

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank: 52 µV

Signal Obtained from LOQ solution (1.0% of target assay concentration): 519µV S/N = 519/52 = 9.98

The limit of quantification (LOQ) for Voriconazole was found to be 10, and for Caspofungin acetate, it was found to be 9.98. These values are within the specified limit of not more than 10, demonstrating that the analytical method can reliably quantify both Voriconazole and Caspofungin acetate at low concentrations with a signal-to-noise ratio (S/N) of almost 10. This indicates the method's suitability for accurate quantification of these compounds in samples.

The main objective of this study was to develop and validate a robust High-Performance Liquid Chromatography (HPLC) method for the simultaneous quantification of voriconazole and caspofungin in both Active Pharmaceutical Ingredients (API) and injectable formulations, adhering to the guidelines set by the International Conference on Harmonisation (ICH).

A thorough literature review revealed that no single method existed for the simultaneous estimation of voriconazole and caspofungin using RP-HPLC. In response, a novel method was developed utilizing a Shimadzu HPLC system, employing HypersilTM Thermo Scientific "ODSC18 columns and a mobile phase composed of Milli-Q water and acetonitrile in a 1:1 ratio.

The validation process encompassed a linear concentration range of 10-50 µg/ml, yielding high correlation coefficients of 0.9993 for voriconazole and 0.9997 for caspofungin acetate. Precision was established, with the %RSD of the peak response from three replicate injections of standard concentrations being below 2%, indicating the method's precision. Accuracy was assessed by calculating the percentage recoveries of the API from dosage forms, ranging from 99.70% to 100.40% for voriconazole and 99.12% to 99.55% for caspofungin acetate, demonstrating accuracy.

Additionally, the method exhibited high sensitivity with low limits of detection (LOD) of 0.003 µg/ml for voriconazole and 0.09 µg/ml for caspofungin acetate. This highlights the method's capability to detect and quantify these compounds even at very low concentrations. The analysis of pharmaceutical formulations using this RP-HPLC method showed that it is suitable for routine analysis, with minimal interference from typical additives found in pharmaceutical formulations. In summary, this proposed RP-HPLC method is straightforward, sensitive, reliable, and can be applied for the simultaneous determination of voriconazole and caspofungin acetate in bulk samples and pharmaceutical formulations as required for specific analytical needs and situations.

Conculsion

A simple, specific, and highly accurate reverse phase high-performance liquid chromatography (HPLC) method has been successfully developed for the simultaneous quantification of Voriconazole and Caspofungin acetate in both bulk samples and pharmaceutical formulations. This method can accurately and precisely determine the concentrations of these compounds, making it suitable for routine analysis in both individual drug substances and formulations. The method has been rigorously validated in accordance with ICH Q2 (R1) guidelines, ensuring its reliability and suitability for use by pharmaceutical industries.

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