Research Article

Analytical Method Development and Validation of Posaconazole by QbD Approach

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ABSTRACT

Quality by design (QbD) is modern and systematic approach for product development and quality of pharmaceuticals. In this concept of QbD it is essential to define desire product performance profile, analytical target Profile and identify critical quality attributes throughout designing and development of a process. This review aimed to identify implementation of QbD in analytical procedure validation. Integration of QbD is an essential step in developing a systematic method which effectively fit for purpose and assures quality. The outcomes of organized analytical process development and validation are understandings of critical quality attributes, risk assessment and outlining design space. Consequently, control strategy can be set for further investigation or other issues. Posaconazole is a approved lipophilic triazole antifungal agent that exhibits potent and broad- spectrum antifungal activity in vitro and in vivo against most Candida spp., Cryptococcus neoformans, Aspergillus spp., many Zygomycetes, endemic fungi and dermatophytes. It has been documented that posaconazole has potency and spectrum of activity similar to those of itraconazole and superior to those of fluconazole against clinically important isolates of Candida spp., C. neoformans and Aspergillus spp. A spectrophotometric method has been developed and validated for the determination of Posaconazole in pharmaceutical formulation. QbD approach was carried out by varying 19 parameters and critical parameters were extracted by using principal component analysis and by observation. The extracted critical parameters are summarized in Table 7.1. PCZ followed linearity in the concentration range of 10–50 g/mL. The proposed method was applied for pharmaceutical formulation and percentage label claim was found to be 99.05%. The amount of drug estimated by proposed method was in good agreement with the label claim. The method was found to be precise as indicated by the inter-day and intra-day analysis showing % RSD less than 2. There was no any interference of excipients showing that the method was specific. Limit of detection and limit of quantitation were 0.09 and 0.27 g/mL, respectively.

Keywords: Posaconazole, Analytical Process Development, QbD, UV spectrophotometry, ICH guidelines. Process Validation.

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INTRODUCTION

Quality by design (QbD) is modern and systematic approach for product development and quality of pharmaceuticals. Pharmaceutical quality can be assured by understanding as well as controlling formulation and manufacturing variables through such structured context. Now-a-days the concept of QbD can be extended to analytical techniques. In this concept of QbD it is essential to define desire product performance profile, analytical target Profile and identify critical quality attributes throughout designing and development of a process. This review aimed to identify implementation of QbD in analytical procedure validation. Integration of QbD is an essential step in developing a systematic method which effectively fit for purpose and assures quality. The outcomes of organized analytical process development and validation are understandings of critical quality attributes, risk assessment and outlining design space. Consequently, control strategy can be set for further investigation or other issues[1].

Moreover, it also meets FDA requirements for pharmaceutical quality management system. Posaconazole is a approved lipophilic triazole antifungal agent that exhibits potent and broad- spectrum antifungal activity in vitro and in vivo against most *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., many *Zygomycetes*, endemic fungi and dermatophytes. It has been documented that posaconazole has potency and spectrum of activity similar to those of itraconazole and superior to those of fluconazole against clinically important isolates of *Candida* spp., *C. neoformans* and *Aspergillus* spp. This new triazole has been developed for the treatment of fungal infections, which most often occur in severely immunocompromised patients, such as organ transplant patients or cancer patients undergoing chemo-therapy. Since posanconazole has low solubility in aqueous and acidic media, its absorption is dose limited and significantly dependent upon food intake. The time to reach the maximum plasma concentration has been reported to be 5–8 hours following oral administration of a single dose. The aim of the method is to development and validation for the determination of Posaconazole in pharmaceutical formulation[2].

MATERIALS AND METHODS

Materials

Posaconazole reference standard (99.5%) and raw material were purchased from SimSon Pharma Limited, Mumbai. Methanol analytical grade was provided by Vinca Traders, Indore.

Instrumentation

A Shimadzu (Kyoto, Japan) double beam spectrophotometer, 1800 model with 1 cm quartz cells was used to perform the absorbance measurements. Prior to use, background correction was carried out using the matrix solvent (methanol). For the analysis, the wavelength was set at 260 nm.

Experimental

Standard and sample preparation

Posaconazole reference standard was accurately weighed and dissolved in a 200 mL volumetric flask using methanol to yield the concentration of 25.0 µg/ml. An aliquot of this solution was diluted to 10.0 µg/mL using the same solvent. The raw material was also accurately weighed and transferred to a 100 mL volumetric flask. It was completed with methanol until reach the concentration of 50.0 µg/mL.From this solution, it was prepared a 10.0 µg/ml one using the same solvent[3].

Spectrophotometric Conditions

For the selection of analytical wavelength, standard solution of PCZ was scanned in the spectrum mode from 400 nm to 200 nm. From the spectrum, λ max of PCZ, 260 nm (Figure 1) was selected.

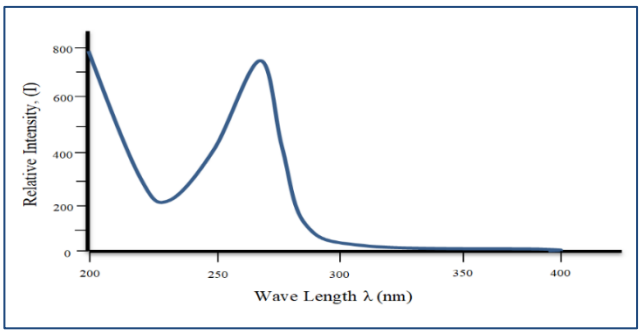

Figure 1: UV Spectrum of Posaconazole

Determination of Variable Parameters for Method

According to QbD approach, the first step is to determine the variable parameters method. Thus, the variable parameters spectrophotometric method was designed as Ishikawa diagram (Figure 2).

For all the variable parameters as stated in Ishikawa diagram, the absorbances were recorded over the concentration range according to respective method. Working solution (50 μg/mL) was scanned from 400 to 200 nm and two peaks were observed at wavelengths 210 nm, and 260 nm. These two wavelengths were used as variable parameters. Also, the solubility was studied in various solvents including distilled water, 0.1 N NaOH, 0.1 N HCl, and methanol. The sharpness of spectra was compared for selection of critical parameter. Scan speed was varied as fast, medium, slow, and very slow over the range 400– 200 nm, while slit width and sampling interval were varied in particular ranges of 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 nm and 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 nm, respectively[4].

For the estimation of PCZ, two types of sample preparations were selected and evaluated. Tablets were formulated as per the master formula and were used in method development. Average weight of tablets was noted and tablets were triturated. Tablet powder equivalent to average weight was taken for study. Matrix containing excipients like lactose monohydrate, microcrystalline cellulose, and corn starch was mixed with PCZ and evaluated for the method development. Recovery study was carried out at three levels 80%, 100%, and 120%[5,6].

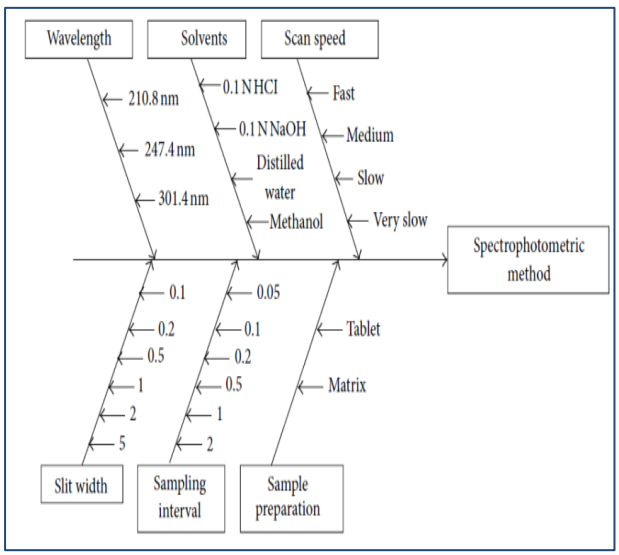

Figure 2: Fishbone Diagram for Spectrophotometric method

Extraction of Critical Parameters

From the evaluated variable parameters, critical parameters were extracted by two ways, observation and principal component analysis using SPSS software. By comparing the spectral shape, sharp- ness, and absorbance of linearity and range, few parameters were selected as critical parameters. In principal component analysis, all these parameters were entered in variable entry window of SPSS software. Simultaneously, all the values of variable parameters were arranged in a datasheet. This datasheet was then substituted in the data entry window of SPSS software. Then the program was run to get principal components (critical parameters)[7,8].

Table 3: Critical Parameter

Critical Parameter Extracted					
By Observation			By Principal Component analysis		
Sr. No.	Parameter	Extracted Result	Sr. No.	Parameter	Extracted result
1.	Solvent	Methanol	1.	Wave length	260 nm
2.	Sample preparation	Tablets	2.	Scan speed	Medium
3.			3.	Slit width	1.0
4.			4.	Sampling interval	0.2

Validation

Validation of the developed method was done according to the USP 2006, Asian edition.

Linearity and range

The linearity was determined by analyzing 6 independent levels of calibration curve in the range of 4-14µg/ml. Absorbance of each solution against methanol was recorded at curve of absorbance vs concentration was plotted and correlation co-efficient and regression line equation for Posaconazole were determined.

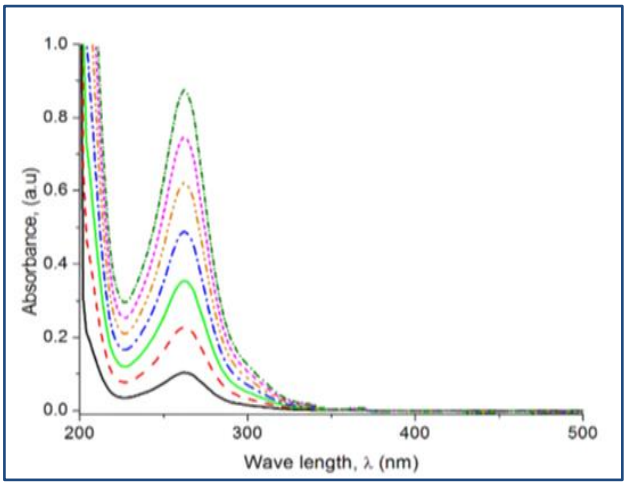

Figure 3: Overlay Spectra of Posaconazole

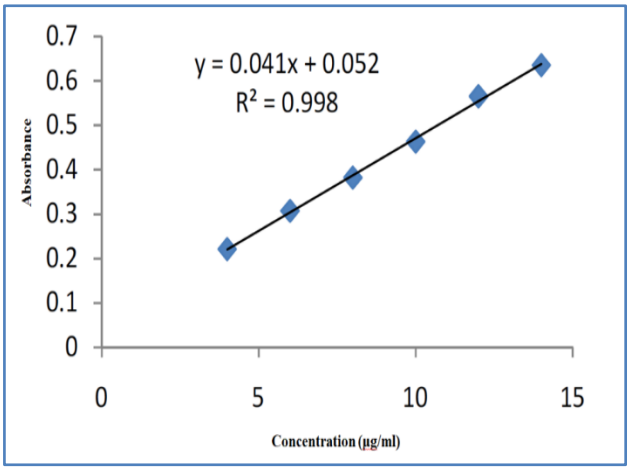

Figure 4: Calibration Curve of Posaconazole

Table 5: Calibration Data of Posaconazole

Sr no.	Concentration (µg/ml)	Absorbance mean ± S. D. (n=5)	% C. V.
1	4	0.210±0.0018	0.85
2	6	0.307±0.0022	0.74
3	8	0.382±0.0014	0.39
4	10	0.464±0.0027	0.57
5	12	0.563±0.0042	0.74
6	14	0.630±0.0058	0.92

Precision

Intra-day precision was determined by analyzing Posaconazole (4-14 µg/ml) at three different time points of the same day and inter-day precision was determined by analyzing Posacoanzole (4-14 µg/ml) at three different time points on different days and %RSD was calculated.

Intraday precision

%RSD was found to be in the range of 0.27-1.19.

Table 6: Result of Intraday precision

Concentration (µg/ml)	Absorbance mean ± S. D. (n=3)	% C. V.
4	0.211±0.0025	1.19
8	0.383±0.0015	0.39
12	0.565±0.0015	0.27

Interday precision

%RSD was found to be in the range of 0.26-0.98

Table 7: Result of Interday precision

Concentration (µg/ml)	Absorbance mean ± S. D. (n=3)	% C. V.
4	0.212±0.0015	0.72
8	0.381±0.0010	0.26
12	0.561±0.0055	0.98

Accuracy

Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug in the pre-analyzed powder for infusion

samples within the analytical concentration range of the proposed method at three different set at level of 80%, 100% and 120%. The amount of Posaconazole was calculated at each level and % recoveries were computed.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity.

LOD= 3.3*σ/S and LOQ= 10*σ/S

Where, σ = the standard deviation of y-intercepts of regression lines

S = the slope of the calibration curve

RESULTS AND DISCUSSION

Implementation of QbD approach was carried out by studying variable parameters in the analytical method development. Critical parameters were extracted by observation of results as well as performing principal component analysis. Also, method was validated according to ICH Q2 (R1) guidelines.

A spectrophotometric method has been developed and validated for the determination of Posaconazole in pharmaceutical formulation. QbD approach was carried out by varying 19 parameters and critical parameters were extracted by using principal component analysis and by observation. The extracted critical parameters are summarized in Table 7.1. PCZ followed linearity in the concentration range of 10–50 g/mL. The proposed method was applied for pharmaceutical formulation and percentage label claim was found to be 99.05%. The amount of drug estimated by proposed method was in good agreement with the label claim. The method was found to be precise as indicated by the inter-day and intra-day analysis showing % RSD less than 2. There was no any interference of excipients showing that the method was specific. Limit of detection and limit of quantitation were 0.09 and 0.27 g/mL, respectively. The result did not show any statistical difference between different solvents and different wavelengths suggesting that the method developed was robust.The statistical data of validation is summarized in Table 9.1

Table 8: Summary of Validation Parameter

Sr. No.	Parameter		Results
1	λmax		260 nm
2	Regression line equation		Y=0.0418X+0.0528
3	Correlation coefficient (R^2)		0.9981
4	Precision (%RSD)	Intraday Precision	0.27-1.19
		Interday Precision	0.26-098
5	Accuracy (% Recovery)		99.11-101.18
6	LOD		0.146 µg/ml
7	LOQ		0.443 µg/ml

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CONCLUSION

The goal of a well-characterized method development effort is to develop a reliable method that can be demonstrated with a high degree of assurance to consistently produce data meeting predefined criteria when operated within defined boundaries.

The UV spectrophotometric method developed demonstrated to be fast, precise and suitable for posaconazole determination. UV spectrophotometry is a very useful technique, already employed for other azole antifungal drugs. This method is a suitable alternative to HPLC in routine quality control.

Implementation of QbD approach resulted in more robust methods which can produce consistent, reliable, and quality data throughout the process and also save time and money.

Analytical methods play an essential role under QbD.

- ✓ Support product and process development.
- ✓ Enable advanced strategies like PAT.

Analytical method development and validation by QbD plays a key role in the pharmaceutical industry for ensuring the product quality. The outcome of AQbD is the understanding from product development to commercial production. Scientist can easily identify the risk initially so that quality can be increased.

AQbD tools are ATP, CQA, Method Optimization and Development with DoE, MODR, and Control Strategy with Risk assessment, Method validation and Continuous Method Monitoring (CMM) and continuous improvement. QbD requires the right ATP and risk assessment and usage of right tools and performing the appropriate quantity of work within proper time lines.

QbD is modern approach being extensively used in pharmaceutical industry than empirical approaches of the product development because it reduces the product variability and risk associated.

Regulatory flexibility is achievable by applying QbD approach to the design of analytical methods but requires a very high degree of understanding and robust quality systems Rather than continuing to perform analytical technology transfer exercises and ICH validation, a QbD approach based on a risk-assessed change control procedure should be adopted. Each time a method is changed, a risk assessment should be performed. Where the change is identified as having a potential to take the method outside its known design space, a method evaluation and, if appropriate, an equivalency exercise

should be performed to ensure method performance criteria are still met. QbD in analytical procedure is in accordance with ICH Q2 (R1), Q8 (R2) and Q9 guidelines. The applications of implementing AQbD in process development and validation are understandings of critical quality attributes, risk assessment and outlining design space. Consequently, control strategy can be set and continual improvement is possible for further investigation or other issues. Moreover, it also meets FDA requirements for pharmaceutical quality management system. The most common outcome is methods are more robust and rugged, and thus survive the challenges of long-term usage of product.

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