An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

QbD APPROACH TO METHOD DEVELOPMENT AND VALIDATION OF ORCIPRENALINE SULPHATE BY HPLC

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ARTICLE INFO

Key Words

Quality by Design, HPLC, Validation, Orciprenaline sulphate, Design approach.

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

A simple, precise, rapid and accurate QbD approach to HPLC method was developed and validated for Orciprenaline sulphate as per the ICH Guidelines. Quality by design (QbD) refers to the achievement of certain predictable quality with desired and predetermined specifications. A very useful component of the QbD is the understanding of factors and their interaction effects by a desired set of experiments. The Central Composite Design was selected and two factors; Mobile phase ratio and pH was analyzed at three different level. The measures responses were Retention time, Theoretical Plates and Peak asymmetry. The method was carried out on Phenomenex ODS C-18 column (150mm X 4.6mm and 5µm) using mixture of 0.01% TEA in water: ACN (82:18 v/v), pH is 7.0. The Flow Rate was adjusted at 1ml/min and Detection in UV Detector. The Retention Time of Orciprenaline sulphate was found to be 4.767 minutes. The Calibration Curve was found to be linear in the range 10-200µg/ml with Correlation Coefficient (R^2=0.997). The Limit of Detection and Limit of Quantitation were found to be 0.10µg/ml and 0.31µg/ml respectively. The %Recovery of Orciprenaline sulphate was found to be in range of 100.02±0.40 % to 100.05±0.50%. The proposed method was successfully applied for the estimation of Orciprenaline sulphate Marketed tablet Formulation.

INTRODUCTION

Quality by Design (QbD)[1-4]is well established in the pharmaceutical industry for manufacturing processes (ICH Q8 for pharmaceutical development and ICH Q11 for development and manufacture of drug substances). QbD is "a systematic approach to development that begins with predefined objectives and emphasizes understanding and control, based on sound science and quality risk management". The outcome of using QbD concepts is a well-understood product and process that consistently delivers its intended performance. The knowledge obtained during development may support the establishment of a design space and determines suitable process controls. This same QbD principle has been applied to the development of analytical methods and is termed "Analytical QbD" (AQbD). Analogous to process QbD, the outcome of AQbD is well understood, fit for purpose, and robust method that consistently delivers the intended performance throughout its lifecycle. High performance liquid chromatography (HPLC) [5-6] is a type of column chromatography used frequently for analytical chemistry and biochemistry. RP-HPLC is the choice for the majority of samples. It consists of a non polar stationary phase and an aqueous, moderately polar mobile phase. The quality of HPLC methods has become increasingly important in a QbD environment. For the purpose of QbD for HPLC methods, robustness and ruggedness should be verified early in the method development stage to ensure method

performance over the lifetime of the product. Otherwise, if a non-robust or non-rugged method is adapted, significant time and resource may be required to redevelop, revalidate and retransfer analytical methods. Literature survey reveals that QbD approach HPLC method for Piracetam[7] ; Artemether And Lumefantrine in combined dosage form[8] ; Fluoxetine Hydrochloride And Olanzapine In Pharmaceutical Dosage Form[9] ; were reported. The present work is aimed todevelop QbD approach to HPLC method development and validation of Orciprenaline Sulphate.

- The principles of Quality by Design (QbD) are starting to be applied to analytical methods across the pharmaceutical industry. QbD concepts is resulting in an enhanced focus within the industry on improving robustness and productivity of manufacturing processes, whilst enhancing the required quality. A very useful component of the QbD is the understanding of factors and their interaction effects by a desires set of experiments.

- So far to our present knowledge on QbD approach to Analytical method development and validation by HPLC is not available in the literature for analytical method development of Orciprenaline sulfate.

- To establish a validated test method as per ICH guidelines for the determination of assay of Orciprenaline Sulphate by RP-HPLC.

MATERIALS AND METHODS

Instruments and Reference Standards: HPLC system, FT-IR (Shimadzu), Double Beam UV Spectrophotometer (Shimadzu UV1800), Pure sample of Ceftriaxone Sodium.

Methodology

Preparation of Reference Standard Solution: The standard stock solution was prepared by dissolving 10 mg of Orciprenaline Sulphate in 10 ml methanol (1000 µg/ml) A 10 µg/ml was prepared by diluting 0.1ml of stock solution to 10 ml with methanol.

Selection of detection wavelength: The Solution of Orciprenaline Sulphate was prepared in mobile phase solution having concentration 10µg/ml and the detection of wavelength was carried out by scanning in the range of 200-400nm.

Method development by QbD approach

1. Define method intent[9-10]: The goals of HPLC method development have to be clearly defined, as pharmaceutical QbD is a systemic, scientific, risk based, holistic and proactive approach that begins with predefined objectives and emphasizes product and process understanding and control.

2. Perform experimental design[11-12]: A systematic experimental design is needed to assist with obtaining in- depth method understanding and performing optimization. Here an efficient and comprehensive experimental design based on systematic scouting of two key components of the RP-HPLC method (mobile phase and pH) is presented. It forms a chromatographic database that will assist with method understanding, optimization and selection. In addition, it can be used to evaluate and implement change of the method, should it be needed in the future, for example should the chromatographic column used no longer be commercially available, or an impurity.
is no longer relevant.

Factorial Design: Central composite statistical screening design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the in-vitro release of the drug. A 2-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with

Design Expert® (Version 11.0, Stat-Ease Inc., and Minneapolis, MN).

$$Y = \beta0 + \beta1A + \beta2B + \beta12AB + \beta11A2 + \beta22B2$$

Where Y is the measured response associated with each factor level combination; β0 is an intercept; β1 to β22 are regression coefficients computed from the observed experimental values of Y from experimental runs; and A & B are the coded levels of independent variables. The terms AB, A2 and B2 represent the interaction and quadratic terms, respectively. The factors were selected based on preliminary study. Mobile phase composition (A) and pH (B) were selected as independent variables. The

Retention time, peak area and peak asymmetry were selected as dependent variables.

3. Evaluate experimental results and select final method conditions [13]

These method conditions were evaluated using the three tiered approach. At the first level, the conditions were evaluated for peaks symmetry, retention time and peaks tailing. This resulted in different chromatographic conditions for Ceftriaxone Sodium. The best suited experimental conditions shall be optimized using design expert software.

4. Perform risk assessment with robustness and ruggedness evaluation [14-15]

As the final method is selected against method attributes, it is highly likely that the selected method is reliable and will remain operational over the lifetime of product. Therefore, the evaluation of method robustness and ruggedness to be carried out as the final step of method development is mainly for the method verification and finalization. A risk based approach based on the QbD principles set out in ICH Q8 and Q9 was applied to the evaluation of method robustness and ruggedness. Structured methodologies for risk assessment, such as Fishbone diagram can be implemented to identify the potential risk of the method due to a small change of method parameters or under a variety of conditions such as different laboratories, chemicals, analysts, instruments, reagents, days, etc.

5 Define analytical method performance control strategy: As a result of robustness and ruggedness studies, the overall method understanding of method performance under various conditions can be improved and an analytical method performance control strategy along with appropriate system suitability criteria can be defined to manage risk and ensure the method delivers the desirable method attributes. If the risk is high and is hard to manage, it is an opportunity for the analyst to go back to the database described in experimental design to find a more appropriate method and to go through the procedure as described to ensure method robustness and ruggedness.

Analytical method validation: Validation is documented evidence, which provide a high degree of assurance for specific method. Validation is analytical process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirement for intended analytical application.

Validation of Proposed HPLC Method: The proposed HPLC method was validated as per ICH Q2 (R1) guidelines.

1. Linearity

- The linearity of Orciprenaline Sulphate was determined by analyzing 5 independent levels of calibration curve in the concentration range of 10-200µg/ml in terms of slope, intercept and correlation coefficient values. The calibration curve was prepared by plotting peak area verses concentration and correlation coefficient was determined.

2. Precision

A. Repeatability
- Measure Peak Area of standard stock solution of Orciprenaline Sulphate of 100µg/ml at 280nm.The peak area of the solution was measured 6 times and %RSD was calculated.

B. Intra-Day Precision
- Variation of the results within same day is called intra-day precision. The intra-day precision was determined by analyzing Orciprenaline Sulphate 100, 150 and 200 µg/ml concentrations, three times on same day at interval of 1 hour, simultaneously and %RSD was calculated.

C. Inter-Day Precision
- Variation of results amongst day is called inter-day precision. Inter-day precision was determined daily by analyzing Orciprenaline Sulphate 100, 150 and 200 µg/ml concentrations, for three days and %RSD was calculated.
- The %RSD should be less than 2.

3. Accuracy

- Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage Recovery of

Orciprenaline Sulphate was found out. Recovery between 98-102% justifies the Accuracy method.

4. LOD and LOQ

LOD was calculated out by using following Formula:

5. Robustness: Robustness of the method was determined by subjecting the method to slight change in method condition, Pump Flow Rate and pH and %RSD was calculated.

6. System Suitability Studies: The system suitability was evaluated by six replicate analyses of Ceftriaxone Sodium. The column efficiency and peak asymmetry, Theoretical Plates were calculated for standard solutions.

7. Assay: Weigh and powdered 20 tablets. Weigh an accurately about 100mg of Orciprenaline Sulphate in 100ml of volumetric flask. Add 25ml of diluent, sonication for 5min and make up to the mark with diluent. Filter the final solution with 0.42µ Whatman filter paper. Withdraw 0.1ml of filter solution make up to 10ml analyzed by develop method. Mixed the 5 container of marketed formulation and from that withdraw the powder equivalent to Orciprenaline Sulphate.

RESULTS AND DISCUSSION

Optimization of mobile phase: The mobile phase was successfully obtained after the many trials shown in the below table. Initially mobile phase composed of methanol: water (50:50, v/v) was tried but long retention proper peak was not observed. The peak splitting was observed in mobile phase Acetonitrile: water (20:80, v/v). A satisfactory separation and peak symmetry for the drug was obtained with mobile phase consisting of The mobile phase 0.01% TEA in water: acetonitrile (80:20, v/v) at 1 ml/min flow rate and ambient temperature. Optimization of various parameters for Analysis of Orciprenaline Sulphate using HPLC (By Using Central Composite Design)

Method Validation

System Suitability: The system suitability test was applied to a representative chromatogram to check the various parameters such as Theoretical plates (N) were 8795; Tailing factor (A_S) was 1.05 and % RSD of six replicate injections were 0.26.

Linearity: The calibration curves constructed for Orciprenaline Sulphate were linear over the concentration range of 10-200 µg/ml. Peak areas of analyte was plotted against concentration and linear regression analysis performed on the resultant curve. Typically, the regression equation for the calibration curve was found to be y= 6785x + 19239 with 0.997 correlation coefficient.

Precision

The % RSD for repeatability of peak area measurement for Orciprenaline Sulphate based on six times measurement of same concentration (100µg/ml) was found to be less than 0.026. Interday and Intraday Precision was shown in table.

Recovery/Accuracy: It was done by Recovery Study. Sample solutions were prepared by spiking at 3 levels i.e. 80%, 100% and 120%**.** Percent Recovery data obtained by the proposed HPLC method are shown in Table 9.

Robustness: Robustness of the method was determined by subjecting the method to slight change in method condition like pH of mobile phase and flow rate using 100µg/ml solution of Ceftriaxone Sodium. No significant effect was observed on system suitability parameters such as capacity factor, resolution and theoretical plates of respective components and % RSD was found to be 0.021-0.048%.

LOD and LOQ: The LOQ and LOD were determined based on signal-to-noise ratios, using an analytical response of 10 and three times of the background noise, respectively. The LOD and LOQ were 0.10µg/ml and 0.31µg/ml respectively.

Assay: The single peak at retention time 5.17 min for Orciprenaline Sulphate was observed in the chromatogram of the drug samples extracted from tablets. Experimental results of the amount of Orciprenaline Sulphate in tablets, expressed as percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any excipients, which are normally present in tablets. The drug content was found to be 98.73 ± 1.56 (n=3) for Orciprenaline Sulphate.

Table 1: Coded values for independent variables

Name of the Factor	Coded values	Level		
		-1	0	+1
Mobile phase composition	A	75:25	80:20	85:15
pH	B	6.5	7.0	7.5

Table 2: Design Summary for optimization

Study Type	Response Surface
Design Type	Central Composite Design
Design Model	Quadratic
Runs	11

Factor	Name	Units	Type	Subtype	Minimum	Maximum
Code					0	1
A	Mobile phase	mL	Numeric	Continuous	75	85
B	pH		Numeric	Continuous	6.5	7.5

Table 3: Evaluation degrees of freedom of design for optimization of analysis of Orciprenaline Sulphate by HPLC Response

Response	Name	Units	Analysis	Minimum	Maximum	Ratio	Model
R1	Retention time	min	Polynomial	1.542	4.795	3.11	Quadratic
R2	Theoretical plates		Polynomial	1178	8795	7.47	Quadratic
R3	Peak assymetry		Polynomial	1.05	1.99	1.90	Quadratic

OPTIMIZED CONDITION OBTAINED: It was obtained by studying all responses in different experimental condition using Design expert 11.0 software.

Table 4: Obtained solution for optimized formulation

Code	Mobile Phase	pH	Retention time	Area	Peak asymmetry	Desirability
O10	82	7.477	4.029	4986	1.5	1.000

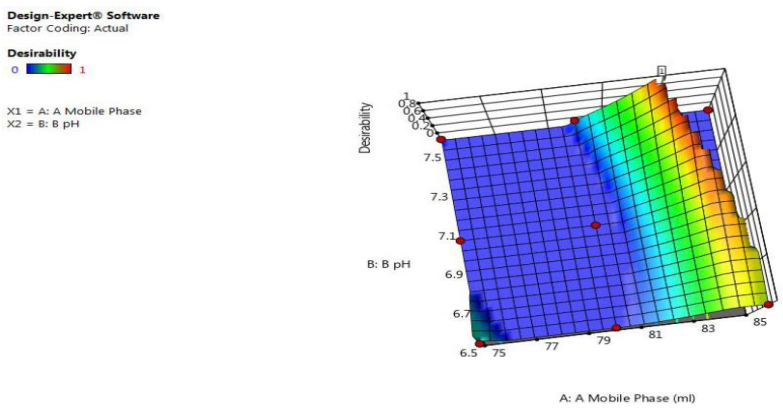

Figure 1: 3D surface plot of desirability for obtaining optimized formulation.

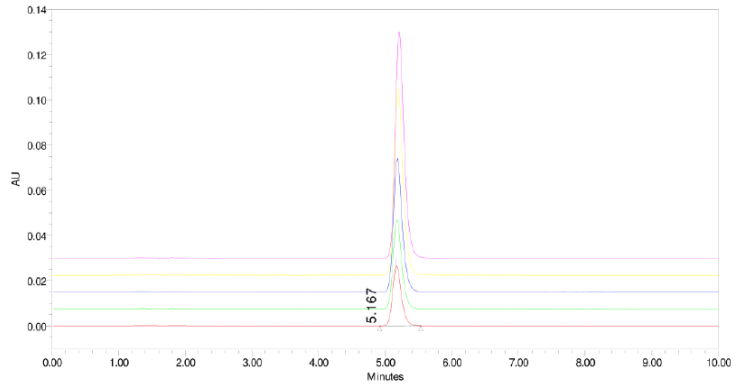

Figure 2: Chromatogram of Linearity of Orciprenaline Sulphate in range 10-200µg/ml

Table 5: Data for Interday and Intraday of Ceftriaxone Sodium

Precision Period	Concentration (µg/ml)	Mean	SD	%RSD
Intra-Day Precision	10	245759	123.62	0.050
	100	552705	308.03	0.056
	200	948739	127.34	0.013
Inter-Day Precision	10	245601	254.94	0.104
	100	552923	108.54	0.020
	200	948561	214.12	0.023

Table 6: Recovery of Orciprenaline Sulphate

Level	Amount from the sample	Amount of standard Orciprenaline Sulphate Spiked	Total Amount (µg/ml)	Recovered amount (µg/ml) ± SD (n=3)	% Recovered Spiked Amount ± SD (n= 3)
Blank	0	50	50		
80%	50	40	90	39.69 ± 0.56	99.25 ± 1.41
100%	50	50	100	49.42 ± 0.48	98.85 ± 0.96
120%	50	60	110	60.57 ± 1.05	100.95 ± 1.75

CONCLUSION

A reversed phase HPLC method development approach using QbD principles has been described. First, the method goals are clarified based on the process understanding. The experimental design describes the scouting of the key RP-HPLC method components including mobile phase and pH. Their interrelationships are studied and optimized conditions are obtained for each combination of mobile phase and pH with the help of design expert 11.0 version. Here a better understanding of the factors influencing chromatographic separation and greater confidence in the ability of the methods to meet their intended purposes is done. Moreover, this approach provides an in-Depth knowledge and enables the creation of a chromatographic database that can be utilized to provide alternative method conditions at a future time should changes to the method be required. Furthermore, the method development is not considered finished until a thorough risk assessment and all the necessary robustness and ruggedness studies are carried out. All the validated parameters were found within acceptance criteria. The validated method is specific, linear, precise, accurate, robust and rugged for determination based on knowledge of method obtained through the method development and the results of risk assessment along with robustness and ruggedness studies,

detailed analytical method performance control strategy can be defined to manage the risk. The approach can be successfully used in laboratory to develop RP-HPLC method for Orciprenaline Sulphate.

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