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APPLICATION OF QUALITY BY DESIGN FOR DEVELOPMENT OF ANALYTICAL RP-HPLC METHOD FOR RANITIDINE HCL

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ABSTRACT

The concept of Quality by Design (QbD) has recently been adopted for the development of pharmaceutical processes to ensure a predefined product quality. Quality by design (QbD) refers to the achievement of certain predictable quality with desired and predetermined specifications. In an attempt to reduce rising development costs and regulatory barriers to innovation and creativity, the FDA and ICH have recently started promoting QbD in the pharmaceutical industry. The present study describes a simple, accurate, precise and cost effective reverse phase high performance liquid chromatographic (RP-HPLC) Method for determination of Ranitidine HCl bulk marketed tablet formulation. The systematic approach, one of the parts of QbD was use for the analytical method development. Detection was done using UV detector at 314 nm. Optimization was done by response surface methodology, applying a three level Box Behenken design with three centre points. Three factors selected were flow rate, pH and Buffer: Acetonitrile concentration in mobile phase composition. The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines for linearity, range, accuracy and robustness. The separation was carried on Phenomenex C18 (4.6 ID mm×150mm; 5µm) with mobile phase 0.02M phosphate Buffer: Acetonitrile (25:75 v/v). Flow rate 0.9 ml/min and at pH 3.0, which was optimized with help of design expert software. High linearity of the developed method was confirmed over concentration range of 10-50 µg/ml and correlation coefficient of 0.9996. The percentage RSD for precision and accuracy of the method was found to be less than 2%. Peak was obtained at retention time of 2.139 min.

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INTRODUCTION

(Part I Basics of Quality by Design)

Quality[1, 2]

Quality is the suitability of either a drug substance or drug product for its intended use. This term includes such attributes as the identity, strength, and purity

Quality is a very important fundamental aspect in our Pharmaceutical Industry. Because, Quality of Medicine can affect life of millions of people within a very short time. Quality is the heart of pharmaceutical industry. Quality is one of the fundamental criteria in addition to safety and efficacy for any entity to be qualified and approved as a drug. For ensuring consistency of performance of pharmaceutical products and systems, the recent emphasis has been on building the "quality" rather than merely testing it. This philosophy forms the basis of Quality by Design (QbD).

Quality by Design (QbD) [1, 2]

Pharmaceutical industries are alert on product Quality, Safety, and Efficacy. Product quality has been increasing by implement scientific tools such as QbD (Quality by Design). Scientific approaches will provide the clear and sufficient knowledge from product development to manufacturing. These QbD tools will minimize the risk by increasing the output and quality. Nowadays QbD approach has been successfully implemented in common formulation development. USFDA has released specific QbD guidance for immediate and extended release drug products as well as biotechnological products. Regulatory authorities are always proposing the implementation of ICH quality guidelines Q8 to Q11.

According to ICH Q8 guidelines, QbD is defined as , "A systematic approach to development that begins with predefined objectives & emphasizes product, process understanding & process control, based on sound science & quality risk management."[4] It means that, design & develop the formulation & manufacturing process to make sure predefined product quality. It requires an understanding of how product & process variables influence product quality. It is a systematic process to build the quality in to final product. QbD requires identification of all critical quality attributes and process parameters as well as determining the level to which any variation can impact the quality of the final product. QbD is all about designing an appropriate process and understanding process performance for the desired product performance. Major element in the overall scheme is continuous improvement, which in turn is based on the knowledge gained during process understanding. The concept gravitates towards a „desired state" marked with „regulatory flexibility" focusing on scientific knowledge building, superior design, demonstration of performance, Quality Risk Assessment (QRM), Design of Experiments (DOE), Process Analytical Technology (PAT) tools, continuous improvement and learning, and life-cycle management. Figure.1 pictorially depicts the building blocks of a QbD-based progression.

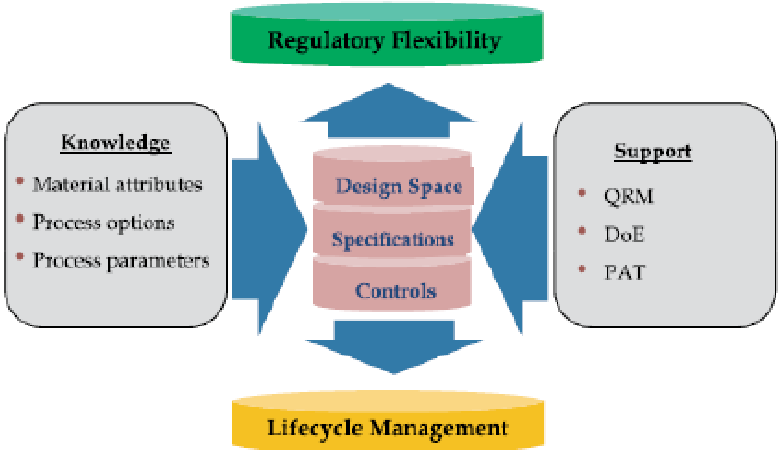

Fig.1 Building blocks of Quality by Design (QbD); Key terms: QRM: Quality Risk Management; DOE: Design of Experiments; PAT: Process Analytical Technology.

Terminology employed during analytical QbD (A QbD)

Quality Target Product Profile (QTPP) [3]

The quality target product profile forms the basis of design for the development of the product. Considerations for the quality target product profile could include:

- Intended use in clinical setting, route of administration, dosage form, delivery systems.
- Dosage strength(s).
- Container closure system.
- Therapeutic moiety release or delivery and attributes affecting pharmacokinetic characteristics (e.g. dissolution, aerodynamic performance) appropriate to the drug product dosage form being developed.
- Drug product quality criteria (e.g., sterility, purity, stability and drug release) appropriate for the intended marketed product.

Quality Attributes

A physical, chemical or microbiological property or characteristic of a material that directly or indirectly impacts quality.

Critical Quality Attributes (CQA) [4]

A CQA is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQAs are generally associated with the drug substance, excipient, intermediates (in-process materials) and drug product.

Critical Process Parameter (CPP) 5

Process parameter whose variability has an impact on critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality.

Process Analytical Technology (PAT) [6]

It is a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e. during processing) of critical quality and performance attributes of raw and in-process materials and processes with goal of ensuring final product quality.

Design Space [7]

The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval.

Control Strategy [8]

A planned set of controls, derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.

A control strategy is designed to ensure that a product of required quality will be produced consistently. Fig.2 describes in short about control space.

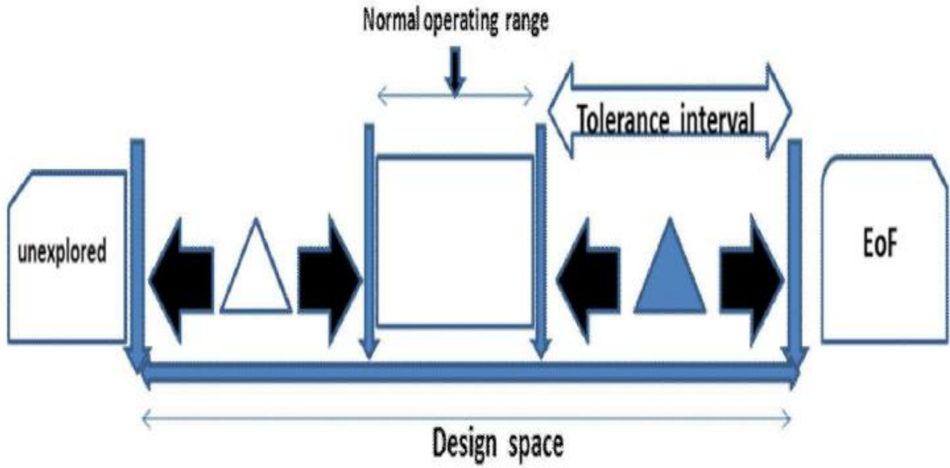

Fig.2 Concept of Design space and control space.

A control strategy can include, but is not limited to, the following:

- Control of input material attributes (e.g. drug substance, excipient, primary packaging materials) based on an understanding of their impact on process or product quality.
- Product specification(s).
- Controls for unit operations that have an impact on downstream processing or product quality (e.g. the impact of drying on degradation, particle size distribution of the granulate on dissolution
- In-process or real-time release testing in lieu of end-product testing (e.g. measurement and control of CQAs during processing).
- A monitoring program (e.g. full product testing at regular intervals) for verifying multivariate prediction models.
- A control strategy can include different elements. For example, one element of the control strategy could rely on end-product testing, where as another could depend on real-time release testing.

Real time Release Testing [6]

The ability to evaluate and ensure the quality of in-process and/or final product based on process data, which typically include a valid combination of measured material attributes and process controls.

Design of Experiment (DOE) (Formal Experimental Design)

It is structured, organized method for determining the relationship between factors affecting a process and the response of that process. It is useful in determination of design space, optimize the product or process.

Elements of pharmaceutical development (REF) [9]

QbD comprises all elements of pharmaceutical development mentioned in the ICH guideline Q8.Pharmaceutical Development section is projected to provide a complete understanding of the product and manufacturing process for reviewers and inspectors. To design a quality product and its manufacturing process to consistently deliver the intended performance of product is the aim of pharmaceutical development. The information and knowledge gained from pharmaceutical development studies and manufacturing experience provide scientific understanding to support the establishment of the specifications, and manufacturing controls.

Linkage of QbD with ICH Q8, Q9, Q10 Guidelines [10, 11]

If we see the basic concept and approach of QbD it is very much linked with ICH Q8, Q9, and Q10 Guidelines. It is described as follows:

QbD Approach Q8 (R2)

- Quality target Product Profile.
- Determine Critical Quality Attributes.
- Link raw material attributes and process parameters CQAs and perform risk assessment
- Develop a Design Space.
- Design and implement a control strategy.
- Manage product life cycle, including continual improvement.

ICH Q9 Quality Risk Management

The purpose of ICH Q9 was to offer a systematic approach to quality risk management. Importantly, it is noted that use of quality risk management can "facilitate, but does not obviate, industry‟s obligation to comply with regulatory requirements and does not replace appropriate communications between industry and regulators". Two important principles were highlighted in this document for the use of Quality Risk Management:

1. The evaluation of the risk to quality should be based on scientific knowledge and ultimately
link to the protection of the patient;

2. The level of effort, formality and documentation of the quality risk management process
should be commensurate with the level of risk.

These are important caveats that should be remembered as risk assessment. It is a process that can easily be overused and lead to large amounts of unnecessary documentation. In Annex 1 to ICH Q9 the following tools are suggested for risk management in the pharmaceutical industry:

- ✓ Flow charts;
- ✓ Check sheets;
- ✓ Process mapping;
- ✓ Cause and effect diagrams;
- ✓ Failure mode effects analysis (FMEA);
- ✓ Failure mode effects and criticality analysis;
  - Fault tree analysis;
  - Hazard analysis and critical control points;
  - Hazard operability analysis;
  - Preliminary hazard analysis;
  - Risk ranking and filtering;
  - Various statistical tools:
    - Acceptance control charts;
    - DOE;
    - Histograms;
    - Pareto charts;
    - Process capability analysis.

ICH Q10 –Pharmaceutical Quality System

Describes a comprehensive model for an effective pharmaceutical quality system that is based on International Organization for Standardization (ISO) quality concepts, includes applicable cGMP regulations, and complements ICH Q8 and ICH Q9.

The Pharmaceutical Quality System had described four key elements:

✓ A process performance and product quality monitoring system;
✓ A corrective action and preventive action system;
✓ A change management system;
(Part-II Application of QbD principles to Analytical Method development)

QbD principles in method development process [9, 12]

Current regulatory guidelines are focused on QbD approach rather than Quality by Testing (QbT) approach. As discussed earlier the FDA and ICH guidelines (Q8, Q9 and Q10) talk about process or product development. But they do not elaborate application of QbD in Analytical Method Development (AMD). As AMD is an integral part of Pharmaceutical development, application of QbD to this area has become essential. Several researchers have attempted to interpret the concepts of QbD in AMD.

Recently, a number of articles have described application of the principles of QbD to the development of analytical methods. Many of the terms used in QbD are very familiar to analytical chemists, including DoE, which has already been extensively used in the development of chromatographic methods.

The steps involved in Analytical QbD method development are as follows.

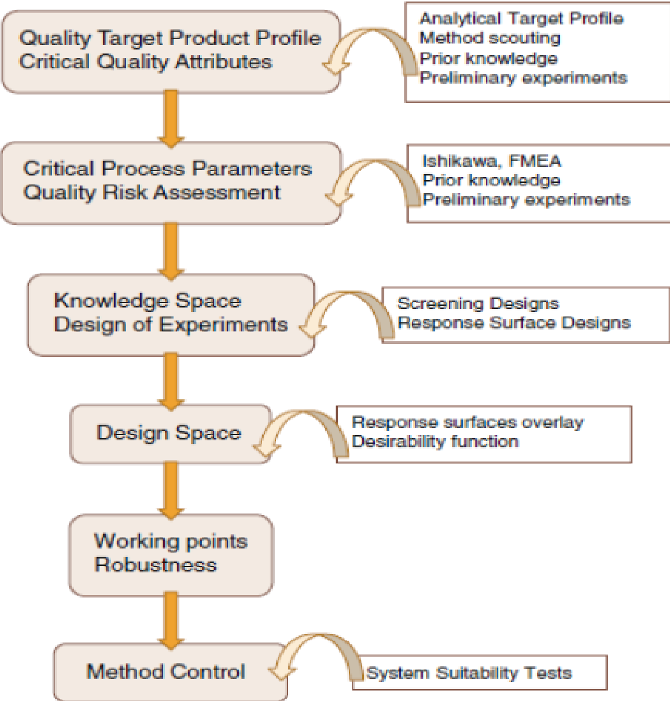

Fig.3 Analytical method development in QbD.

Steps involved in Analytical Method development in QbD

The first step in designing of analytical methods by using the principle of QbD is the selection of the type of analytical method and the various factors affecting the method. These factors can be classified as primary parameters and secondary parameters. This step involves the study of primary parameters. The parameters are then prioritized based on the extent of the effect caused on analysis. This phase is followed by screening phase which calculates approximately the effects of secondary parameters on selected responses (like resolution and selectivity in case of HPLC). The model which can be used for this stage are Two Levels Full Factorial, Two Levels Fractional Factorial, Plackett- Burman. The next step is response surface generation by using any of the method from Central Composite Design, Box- Behnken Design, Full Factorial Design at three levels, Doehlert Matrix Design or D- Optimal design. This stage is followed by the optimization stage which employs the use of computer software as well virtual screening to determine MODS.

This approach when applied to HPLC analytical method development includes four main steps: The first step is to determine primary parameters like screening of column chemistry, organic modifier, pH of buffer and mobile phase. This is followed by next step where the selectivity optimization is confirmed through changes in gradient time and mobile phase temperature. Finally, column geometry optimization to get sufficient resolution and MODS is determined.

A simple example is selection of conditions for chromatographic method development on the basis of structure of drug defining physicochemical properties like logP, logD, pKa etc. The advent of computer technology has reduced the time required for calculation and results are more precise with the use of statistical methods for treatment of data. The various statistical methods used can be Multiple Linear Regressions (MLR), Partial Least Square (PLS) or Principal Component Analysis and other tools like Analysis of Variance (ANOVA), student"s t-test, Pearson coefficient are also used whenever required. Fig. 4 Describes Steps involved in the DOE and designs employed for Screening and Response Surface

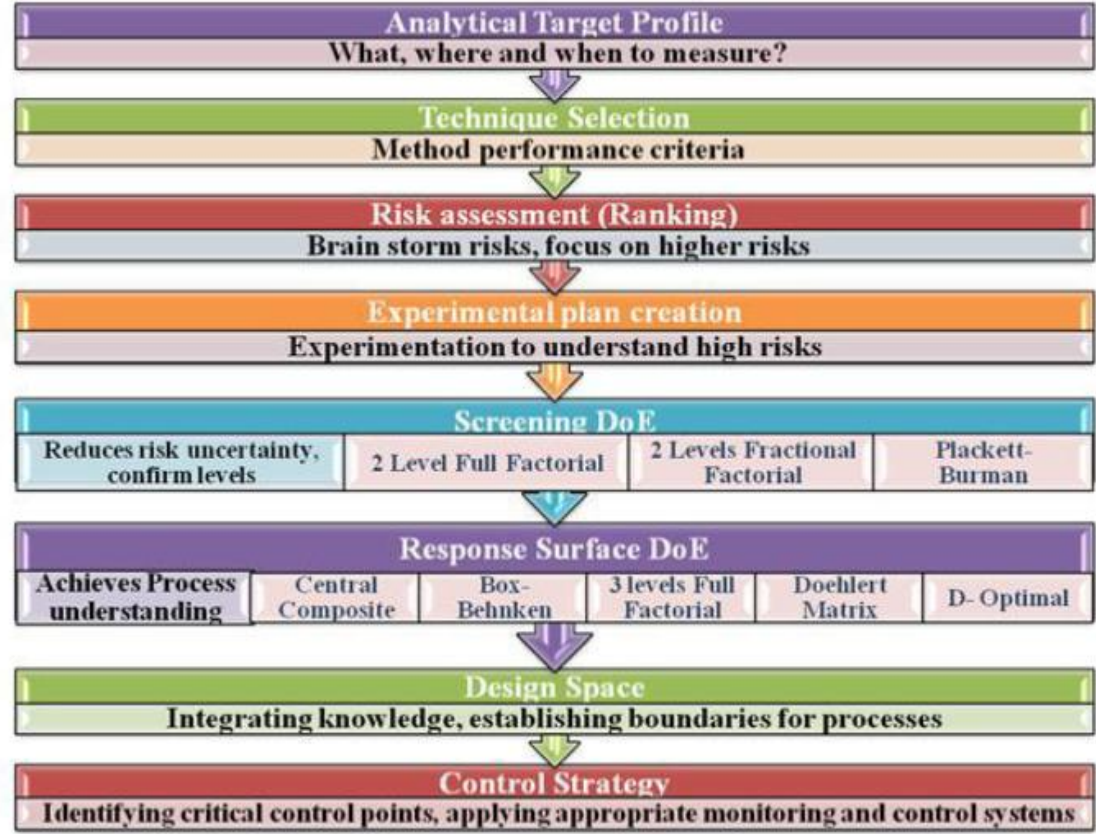

Fig.4 Steps involved in the DOE and designs employed for Screening and Response Surface Steps.

MATERIALS AND METHODS

Reagents and Chemicals:-

Reference Standard and Marketed Formulation of Ranitidine HCL were Obtained from Glaxosmithkline, Nasik.
HPLC grade water, Acetronitrile, Methanol were purchased from Modern Laborotaries Pvt. Ltd., Nasik

Instruments:-

Analysis was performed on HPLC water system equipped with binary LC- 1525 pump and UV-2489 detector. Data acquisition and processing was done using Breeze 2 software, and optimization of method was done Using Design Expert® (Stat-Ease), Ver.10.0. Software.

Selection of Wavelength

Standard solutions of 2-10 µg/mL were prepared in Acetronitrile and were scanned in range of 400-200nm and the Overlain Spectra was obtained. Thus 314nm was selected as a detection wavelength for estimation of drug as shown in Fig. 5

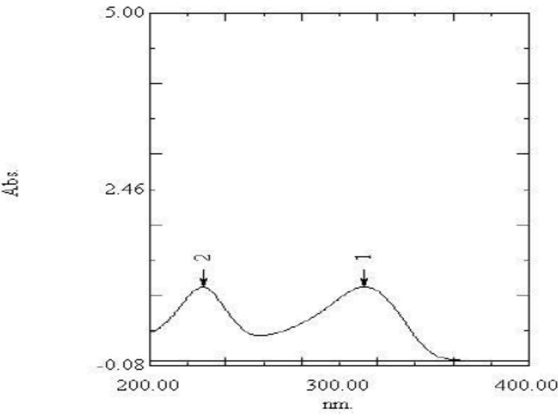

Fig. 5 Overlain Spectra of Ranitidine HCL.

Method Optimization

Initially various mobile phases such as Acetonitrile: water (70:30, v/v, pH adjusted to 3 with ortho phosphoric acid), (0.02 M pot. Dihydrogen phosphate): Acetonitrile: Methanol (40:50:10, v/v), and Acetonitrile: Methanol (70: 30) were tried at flow rate of 0.8ml/min but they didn't produce results. After evaluating the system suitability factors required for analysis; the mobile consisting of Methanol: Water (70:30, v/v) at a flow rate of 0.9 ml/min was selected for further optimization by QbD.

Development of Analytical RP-HPLC Method with Design Space and Control Strategy determination by optimization study:

All the computations for the current optimization study and statistical analysis were performed using Design Expert® software (Design Expert trial version10.0.3.1; State-Ease Inc., Minneapolis, MN, USA).

Application of Design of experiments for method optimization

Design of experiments (DOE-1):

Thus, 32 randomized response surface designs with a Box-Behenken design were used with 17 trial runs to study the impact of three factors on the three key response variables. In this design 3 factors were evaluated, each at 3 levels, and experimental trials were performed at all possible combinations. The flow rate, pH of buffer, mobile phase composition were selected as independent variables and retention time (RT), Theoretical Plate Number (TPN) and Asymmetric Factor were selected as dependent variables based on risk analysis. The resulting data was processed into Design Expert 10.0.3.1 software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to determine the influence of flow rate, pH and mobile phase composition on dependent variables. The probable trial runs using 33 Box-Behnken designs are as shown in table 1. And coded levels are shown in Table No. 2

Table no. 1: 3^2 Box Behenken full factorial design of DoE.

Std	**Run**	**Factor 1 A:Flow Rate mL/min**	**Factor 2 B:Temperature Degree Celcies**	**Factor 3 C:Mobile phase composition ml**	**Response 1 Retention Time min**	**Response 2 Asymmetri c**
2	1	50	0.9	3.8		
9	2	50	0.6	4.6		
1	3	50	0.9	3.8		
7	4	50	0.9	3.8		
8	5	50	0.9	3.8		
13	6	25	0.9	3		
1	7	75	0.9	4.6		
11	8	25	0.6	3.8		
6	9	25	1.2	3.8		
12	10	50	0.9	3.8		
17	11	75	1.2	3.8		
16	12	75	0.6	3.8		
14	13	50	1.2	4.6		
4	14	75	0.9	3		
5	15	50	1.2	3		
3	16	25	0.9	4.6		
15	17	50	0.6	3		

Table no. 2: Translation of coded levels in actual values.

Level of Variable	Concentration of Factors		
	Flow Rate (ml/min)	pH	Mobile Phase Composition ratio (Buffer: Acetonitrile)
Low Level (-1)	0.6	3	50:50
Medium Level (0)	0.9	3.8	25:75
High Level (1)	1.2	4.6	75:25

Application of proposed method for analysis of marketed formulation

Standard stock solution:

Accurately weighed quantity of Ranitidine HCl 10 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with diluent. The resultant solution is used as standard stock solution of Ranitidine HCl. (Concentration 100 dg/ml)

Sample solution preparation:

Accurately weighed tablet powder equivalent to 10 mg of Ranitidine HCl was transferred in a 100 ml volumetric flask and diluent was added. It was shaken vigorously for 5 to 10 minutes. Later the volume was made up to mark with diluent. The solution was filtered through Watman filter paper No.42.

Procedure:

Equal volume (20pL) of standard and sample solutions injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of Ranitidine HCl was calculated by comparing a sample peak with that of standard.

Amount of drug in tablet was calculated using following formula- For Assay of Ranitidine

$$\mathbf{Mg/ml} = \frac{\mathbf{AT \times WS}}{\mathbf{AS \times WT}} \quad \mathbf{X\ 100}$$

Where, AT -Average area of Ranitidine HCl peak in test chromatograms

AS -Average area of Ranitidine HCl peak in standard chromatograms

WS -Weight of Ranitidine HCl working standard taken in mg

WT -Weight of sample taken in mg

Further calculate the amount Ranitidine HCl present in % of Label claim using the following formula:

$$\mathbf{\%\ Label\ Claim} = \frac{\mathbf{Assay\ (mg/ml) \times 100}}{\mathbf{Label\ claim\ in\ mg\ per\ ml\ Ranitidine\ HCl}}$$

Optimized Chromatographic Conditions

The following chromatographic conditions were established by trial and error as shown in Table no. 3 and were kept constant throughout method.

Table No.3:- Optimized Chromatographic Conditions.

Parameter/condition	Description
Column name	Phenomenex C 18
Detector	Waters 2489 (UV-Visible Detector)
Injection Volume	20 pl
Wavelength	314 nm
Mobile Phase	Buffer: Acetonitrile
Programme	Isocratic

Validation of proposed method

System Suitability:

System suitability test is a pharmacopoeia requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard drug solution

Linearity and range:

The linearity of the developed method was estimated using standard solutions of seven different concentrations in the range of 20 to 100pg/ml Each solution was injected in triplicate. A graph of average area vs. concentration was plotted and regression coefficients were calculated.

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

$$\textbf{LOD} \quad \frac{\textbf{3.3x SD}}{\textbf{S}}$$

Where, SD = Standard deviation,

S = Slope of the curve.

Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

It is expressed as the conc. of analyte (e.g., percentage, parts per billion) in the sample.

A typical signal-to-noise ratio is 10:1 or 20:1.

Accuracy (by Recovery method)

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte.

The Relative Standard Deviation should not be more than 2.0%.

Preparation of standard stock solution

10.0 mg of Ranitidine HCl working standard was weighed accurately and transferred into 100 ml volumetric flask, 70 ml of diluent was added and sonicated to dissolve and finally the volume was made with diluents and mixed. The working standard concentration is 100pg/ml. The solution was filtered through 0.45 p Watman filter paper.

Procedure for Preparation of sample Solution:

Prepare the standard solution by taking stock solution equivalent to 50%, 100%, and 150%, each in triplicate. Each concentration injected into the HPLC system, are mention in Table no. 4.

Table No. 4:- Dilution table for Accuracy.

SAMPLE	STOCK SOLUTION-TRANSFER	FINAL VOLUME
Accuracy-80%	0.8	10
Accuracy-80%	0.8	10
Accuracy-80%	0.8	10
Accuracy-100%	0.1	10
Accuracy-100%	0.1	10
Accuracy-100%	0.1	10
Accuracy-120%	1.2	10
Accuracy-120%	1.2	10
Accuracy-120%	1.2	10

Precision

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation, precision Table no. 5 shows amount of sample and dilution.

Method precision:
Determination:

Prepare six different test solution of the 100% test concentration from the same sample matrix. Inject duplicate injections of each test solution.

Table No.5:-Sample Preparation for Precision.

Set No.	Amount of sample added in ml	Amount of diluent in ml
1	3.0	10
2	3.0	10
3	3.0	10
4	3.0	10
5	3.0	10
6	3.0	10

Robustness

It is the measure of capacity of the method to remain unaffected by small but deliberate variation in method parameter and provides an indication of its reliability under normal usage.

Determination:

The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. For example change in physical parameters like pH of mobile phase and its ratio.

Standard preparation, placebo preparation and sample preparation in triplicate were prepared. The sample along with standard and placebo were injected under different chromatographic conditions as shown below.

S **Changes in flow rate. (+0.10ml/min)**

RESULTS AND DISCUSSION

Statistical data analysis (DOE) The layout of actual design of DOE with the subsequent response results are shown in table no.6 as given below.

Table No.6:- Layout of Actual Design of DOE.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	Run	A:MP	B:FLOW	C:PH	RT	ASYM
16	1	50	0.9	3.8	2.473	1.88727
11	2	50	0.6	4.6	2.9	1.0403
13	3	50	0.9	3.8	2.66	1.32158
15	4	50	0.9	3.8	2.468	1.3258
17	5	50	0.9	3.8	2.473	1.88727
5	6	25	0.9	3	2.01	1.16207
8	7	75	0.9	4.6	3.064	1.714
1	8	25	0.6	3.8	3	1.84214
3	9	25	1.2	3.8	2.136	1.83737
14	10	50	0.9	3.8	2.464	1.48368
4	11	75	1.2	3.8	2.111	2.72799
2	12	75	0.6	3.8	3.622	1.33285
12	13	50	1.2	4.6	2.120	1.51851
6	14	75	0.9	3	2.899	1.02602
10	15	50	1.2	3	1.94	2.2218
7	16	25	0.9	4.6	2.44	1.16207
9	17	50	0.6	3	3.011	1.08645

Results for the retention time of DOE:

ANOVA for response surface linear model

The analysis of variance (ANOVA) was performed to identify the significant and insignificant factors. The results of ANOVA for the retention time of DOE are as following Table no.7

Table No. 7 ANOVA table for retention time.

Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	Squares	df	Square	Value	Prob> F	
Model	3.16	9	0.35	12.23	0.0017	significant
A-MP	0.54	1	0.54	18.74	0.0034	
B-FLOW	2.28	1	2.28	79.68	< 0.0001	
C-PH	0.053	1	0.053	1.85	0.2156	
AB	0.10	1	0.10	3.65	0.0977	
AC	0.020	1	0.020	0.68	0.4356	
BC	0.031	1	0.031	1.08	0.3332	
A^2	0.10	1	0.10	3.50	0.1036	
B^2	0.013	1	0.013	0.45	0.5241	
C^2	0.013	1	0.013	0.44	0.5270	
Residual	0.20	7	0.029			
Lack of Fit	0.17	3	0.057	7.87	0.0374	significant
Pure Error	0.029	4	7.272E-003			
Cor Total	3.36	16				

The Model F-value of 14.25 implies the model is significant. There is only a 0.10% chance that a "Model F- Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant.In this case A, B, AB are significant model terms.Values greater than 0.1000 indicate the model terms are not significant.

The "Pred R-Squared" of 0.2972 is not as close to the "Adj R-Squared" of 0.8817 as one might normally expect. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 15.350 indicates an adequate signal. This model can be used to navigate the design space.

Model assessment for the retention time response as dependent variable:

After entering the data in Design Expert software, fit summary applied to data after which "quadratic model" was suggested by the software. According to this model following polynomial equation was obtained. Polynomial equation in coded terms,

The polynomial equation can be used to draw conclusions considering the magnitude of coefficient and the mathematical sign it carries as positive or negative. This polynomial equation shows that, for response that is retention time the main coefficient A, B and interaction coefficient AB, A had a significant effect with p-value less than 0.05.

The equation in terms of coded factors can be used to make predictions about the response forgiven levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. Graphical Presentation: For Retention Time are shown in Fig. 6 and 7

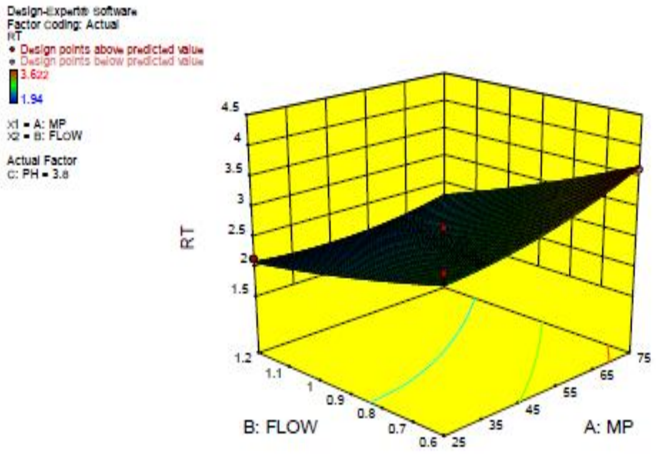

Figure No-6: Response plot of retention time (min) against flow rate and mobile.

Final Equation in Terms of Coded Factors:			
RT	+2.51+0.29*	A-0.52*	B+0.068*C-0.19*AB-0.098*AC+0.088*BC+0.15*A^2+0.027*B^2-

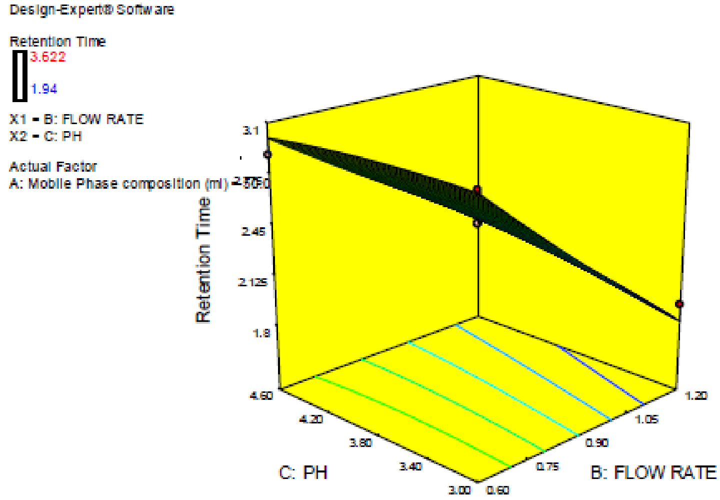

Figure no-7: Response plot of retention time (min) against flow rate and Ph.

Results for the asymmetric factor of DOE:

ANOVA for response surface linear model

The analysis of variance (ANOVA) was performed to identify the significant and insignificant factors. The results of ANOVA for the asymmetric factor of DOE are as following Table no.8

Table No.8: ANOVA table for asymmetric factor.

Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2.87	9	0.32	3.76	0.0474	significant
A-MP	0.079	1	0.079	0.94	0.3655	
B-FLOW	1.13	1	1.13	13.29	0.0082	
C-PH	4.722E-004	1	4.722E-004	5.563E-003	0.9426	
AB	0.49	1	0.49	5.77	0.0473	
AC	0.12	1	0.12	1.39	0.2763	
BC	0.11	1	0.11	1.27	0.2966	
A^2	0.025	1	0.025	0.29	0.6062	
B^2	0.32	1	0.32	3.82	0.0917	
C^2	0.65	1	0.65	7.61	0.0281	
Residual	0.59	7	0.085			
Lack of Fit	0.26	3	0.088	1.07	0.04554	Not significant
Pure Error	0.33	4	0.082			
Cor Total	3.46	16				

The Model F-value of 5.61 implies the model is significant. There is only a 1.66% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than:

Final Equation in Terms of Coded Factors:

Asymmetric factor = +1.58 -0.30*A +0.48*B -0.11*C +0.15*AB -0.032*AC -0.16*BC +0.077*A^2 +0.073*B^2 -0.19*C^2

0.0500 indicate model terms are significant.In this case A, B are significant model terms.Values greater than 0.1000 indicate the model terms are not significant.

The "Pred R-Squared" of 0.4248 is not as close to the "Adj R-Squared" of 0.7216 as one might normally expect. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. A ratio of 9.155 indicates an adequate signal. This model can be used to navigate the design space.

Model assessment for the asymmetric factor response as dependent variable:

The equation in terms of coded factors can be used to make predictions about the response forgiven levels of each factor. By default, the high levels of the factors are coded as +1 and thelow levels of the factors are coded as -1. The coded equation is useful for identifying therelative impact of the factors by comparing the factor coefficients:

Graphical Presentation: For Asymmetric factor are shown in Fig. 8 and 9.

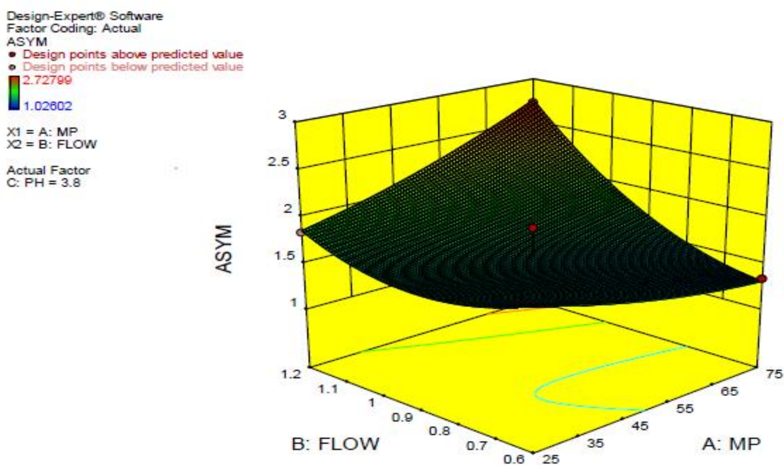

Figure no-8: Response plot of asymmetric factor against flow rate and mobile phase composition.

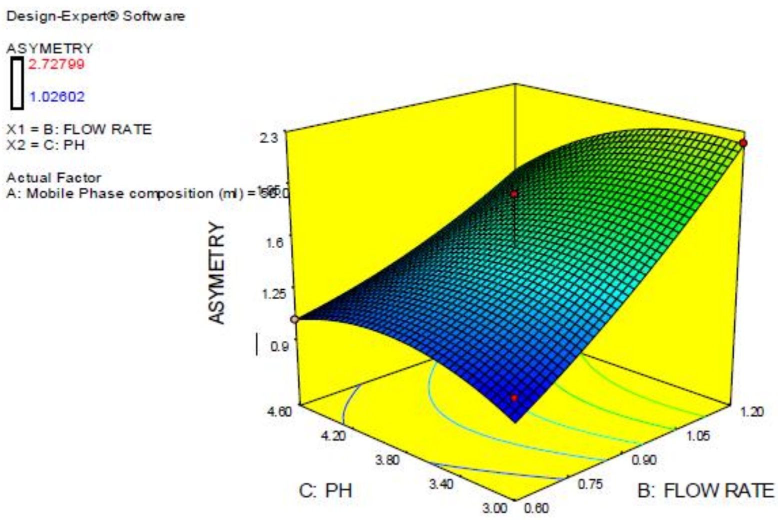

Figure no-9: Response plot of asymmetric factor against flow rate and pH.

DOE optimization result:

The optimization was performed on the basis of response surface modelling by using the numerical and graphical optimization method. Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The numerical optimization finds a point that maximizes the desirability function. The characteristics of a goal may be altered by adjusting the weight or importance. For several responses and factors, all goals get combined into one desirability function. The goal of optimization is to find a good set of conditions that will meet all the goals, the promised optimized method was shown in Table no.9 and results of optimization in Table no. 10.

Table No.9 Proposed optimised method.

Constraints						
		Lower	**Upper**	**Lower**	**Upper**	
Name	Goal	Limit	Limit	Weight	Weight	Importance
A:MP	is in range	25	75	1	1	3
B:FLOW	is in range	0.6	1.2	1	1	3
C:PH	is target = 3	3	4.6	1	1	3
RT	is in range	1.94	2.5	1	1	3
ASYM	is in range	1.02602	2.72799	1	1	3

Optimization solution:

Table No- 10: Result of optimization for DOE.

Number	**MP**	**FLOW**	**PH**	**RT**	**ASYM**	**Desirability**	
1	25.000	0.900	3.000	2.196	1.346	0.949	Selected
2	25.220	0.900	3.000	2.197	1.344	0.949	
3	25.000	0.900	3.008	2.199	1.352	0.947	
4	25.001	0.896	3.002	2.203	1.345	0.946	
5	26.040	0.900	3.000	2.197	1.337	0.946	
6	25.001	0.904	3.000	2.190	1.349	0.946	
7	25.000	0.900	3.020	2.203	1.361	0.944	
8	25.411	0.907	3.000	2.187	1.347	0.942	
9	25.000	0.900	3.028	2.205	1.367	0.942	
10	27.344	0.900	3.000	2.200	1.326	0.941	
11	25.001	0.900	3.042	2.210	1.376	0.938	
12	29.441	0.900	3.000	2.205	1.308	0.934	
13	25.000	0.868	3.000	2.246	1.329	0.926	
14	31.029	0.914	3.000	2.187	1.310	0.914	
15	25.000	0.900	3.137	2.240	1.438	0.912	

16	32.685	0.866	3.000	2.275	1.254	0.898
17	25.000	0.956	3.000	2.112	1.392	0.893
18	46.791	0.887	3.000	2.357	1.187	0.836

Developed Method Operable Design Region.

Design Space for study DOE:
The graphical optimization done by with the help of Design Expert software provided the base to define the design space as shown in following Figure 10

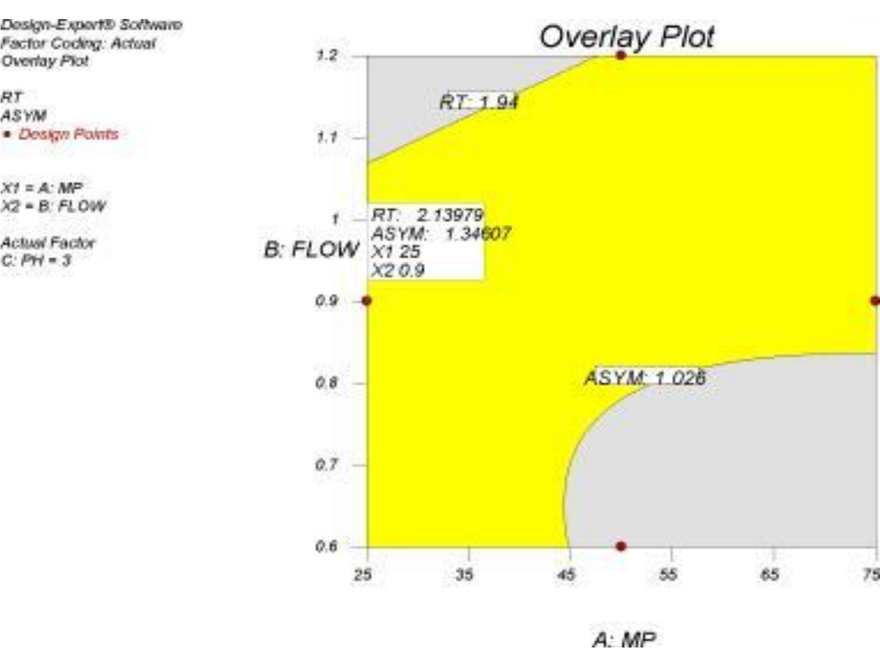

Figure no.10: Design space for DOE.

This plot elaborates that the optimized values of both independent variables in the required target range of retention time & Asymmetric factor lie within the yellow region which is the useful optimum region where the design space can be determined whereas the grey colored region is totally restricted to achieve the target response value of dependent variable, optimized method Table are as follows in Table no. 11

Optimized Method:

Table no-11: Optimized Method:

Flow rate Ml/min	PH	Mobile phase composition (mL)
0.9	3	25:75

Control Strategy
System suitability test are shown in Table no. 12.

Table no-12: System suitability test for Ranitidine HCl.

Sample Name	Retention Time (min)	Area	Plate Count	Tailing Factor
Standard 1	2.020	3874629	3665	1.14
Standard 2	2.000	3898317	3889	1.06
Standard 3	2.187	3905118	3956	1.08
Standard 4	2.173	3831800	4256	1.36
Standard 5	2.189	3811840	3406	1.63
Mean		3864340.8		
S.D		36710.3444		
% RSD		0.94997		

Acceptance Criteria:
1. %RSD of the five replicate injections is NMT 2.0%.
2. Theoretical plates should be more than 2000.
3. Tailing factor should be NMT 2.

Conclusion
1. %RSD of the five replicate injections found to be 0.94997%.
2. Theoretical plates found to be more than 2000.
3. Tailing factor found to be less than 2

Method validation

Accuracy

Table no-13 shows Result and statistical data of accuracy (Ranitidine HCl).

Table no-13: Result and statistical data of accuracy (Ranitidine HCl).

Sr.No.	Conc.Level	Conc. (^g/mL)		Area	Conc. Found (dg/mL)	% Recovery	Average % Recovery	% RSD
		Std.stock solution	Formulation stock solution					
	80%	150	120	656132	268.87	99.59		
1.		150	120	659020	270.17	100.61	100.17	0.42
		150	120	658039	270.87	100.32		
	100%	150	150	936330	298.01	99.33		
2.		150	150	938101	299.01	99.67	99.69	0.21
		150	150	947507	300.22	100.07		
3.	120%	150	180	1262740	320.14	100.04		
		150	180	1257154	319.96	99.98	100.02	0.03
		150	180	1248258	320.18	100.06		

Precision

Table no-14 shows Results of Method Precision of Ranitidine HC1

Table no-14: Results of Method Precision of Ranitidine HC1.

Sr. No.	Concentration (b/ml)	Area	RT (min)	Inj.Vol. (bl)	TP	TF
1	30	656132	2.16	20	6138	1.28
2	30	656132	2.250	20	6285	1.32
2	30	658039	2.27	20	6139	1.33
4	30	658426	2.148	20	6238	1.31
5	30	659020	2.109	20	6240	1.30
6	30	656132	2.27	20	6247	1.29
Mean		657313.5			6214.5	1.305
SD		1331.46			55.94	0.0170
%RSD		0.2			0.9000	1.3086

Acceptance Criteria:

The %RSD for the six determinations shall be NMT 2.0

Conclusion:

The RSD of method precision is 0.9000 %. Therefore, the HPLC method for the determination of Ranitidine HCl is precise.

Linearity:
Table no-15 shows Result and statistical data of linearity of Ranitidine HCl.

Table no-15: Result and statistical data of linearity of Ranitidine HCl.

Sr.no	Concentration (^g/ml)	RT (min)	Area	TP	TF
1	10	2.279	84841	5084	1.50
2	20	2.079	352750	5082	1.34
3	30	2.072	656132	5107	1.34
4	40	2.279	936330	5160	1.16
5	50	2.279	1249955	5219	1.53
	Correlation Coefficient		0.9994		
	Slope (m)		29138		
	Intercept (y)		218141		

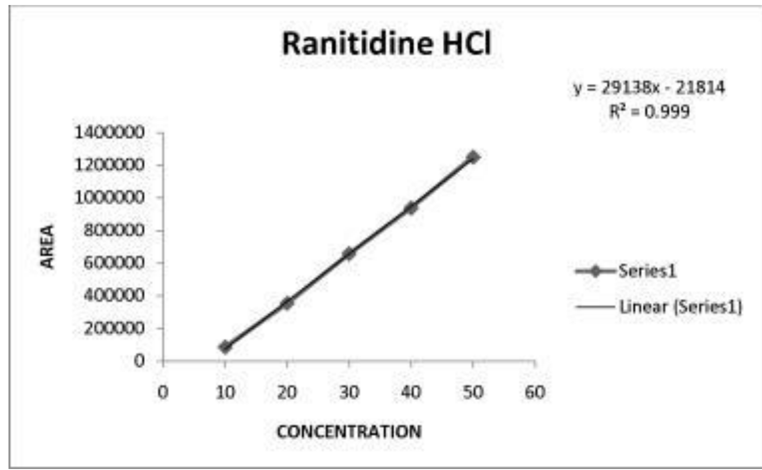

Figure no- 10.15: Linearity graph of Ranitidine HCl.

Detection:
3.6.4.1 Limit of Detection:
It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOD= 3.3 (SD)/S

Where, SD= Standard deviation S = Slope

Calculation of Ranitidine HCl:

$$\text{LOD} = \frac{3.3 \times 1331.46}{29138} = 0.15 \text{ pg/ml}$$

LOD = 0.15 pg/ml
Limit of Detection of Ranitidine HCl is 0.15 pg/ml

Limit of Quantitation:
It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).
LOQ = 10 (SD)/S

S/N = 10/1

Where, SD = Standard deviation
S = Slope

Calculation of Ranitidine HCl

$$LOQ = \frac{10x1331.46}{29138} \quad 0.47\ \mu g/mL$$

LOQ = 0.47 µg/mL
Limit of Quantification of Ranitidine HCl is 0.47 µg/mL

Robustness:

Effect of variation in flow rate of mobile phase Prepared the solution as that of linearity solutions and inject into the HPLC system at flow rate 1.1ml/min. Measured the peak response for the major peaks.
Change in flow rate (±10%) is shown in Table no. 16.

Table no-16: Data for change in flow rate.

Sr.No.	System Suitability parameter	Observations			Limits
		As Such	- 10%	+ 10%	
1	The % RSD of peak area response	0.6318	0.9254	1.1310	NMT 2.0
2	Theoretical plates	6238	6240	6247	NLT 2000
3	Tailing factor	1.31	1.30	1.29	NMT 2.0
4	Retention Time (Min)	2.16	2.08	2.091	

Table No.17 Change in wavelength (±5 nm).

Sr. No.	System Suitability parameter	O	bservations		Limits
		As Such	- 5nm	+ 5nm	
1	The % RSD of peak area response	0.6318	1.0143	0.1836	NMT 2.0
2	Theoretical plates	5434	5246	5150	NLT 2.0
3	Tailing factor	1.15	1.21	1.11	NMT 2.0
4	Retention Time (Min)	2.163	2.169	2.095	

Application of Proposed Method for Estimation of Ranitidine HCl in Marketed Formulation (Assay)

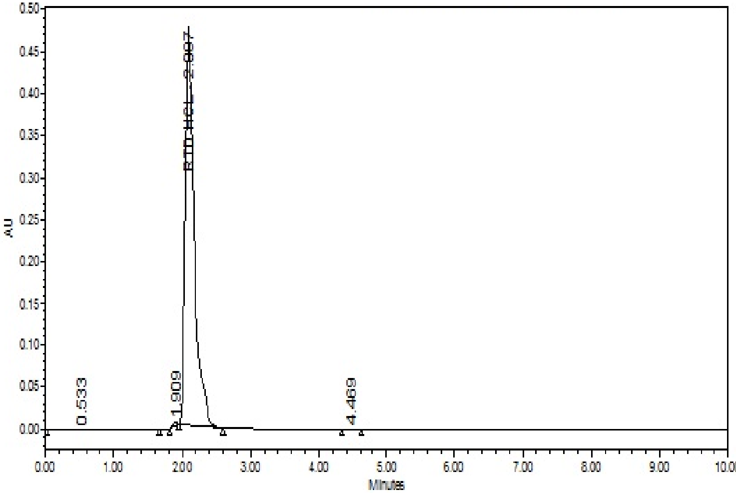

Table no.18: Assay results of marketed formulation.

Sr.No.	Name	RT	Area	USP Plate count	USP Tailing
1	Ranitidine HCl	2.097	555194	5219	1.53

Acceptance criteria
Percentage found should be in the range of 98-102%.

Data interpretation
From the above result, it can be concluded that the assay result is within the limit.

CONCLUSION

A simple, rapid, sensitive, specific, accurate and precise RP-HPLC method has been developed for the first time and optimized utilizing QbD for the determination of BRT and BRZ. The method is rapid as the run time is relativelyshort (10 min) within which the two drugs are well resolved. The main aim of implementing analytical QbD in method optimization was to identify the failures and the critical quality attributes so as to establish a design space such that there is no requirement of revalidation in case of any changes in method parameters. The QbD was applied in HPLC method development so as to verify robustness of the method. The developed HPLC method was suitable for routine quality control analysis.

LIST OF ABBREVIATIONS USED

Selected drug	
RTD HCl	Ranitidine HCl
Chemicals	
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
H2O2	Hydrogen peroxide
ACN	Acetonitrile
Symbols	
Gm	Gram
Mg	Milligram
µg	Microgram
µl	Micro liter
Ml	Milliliter
L	Liter
Nm	Nanometer
λ max	Wavelength of maximum absorbance
Others	
UV	Ultra violet
TLC	Thin layer chromatography
HPTLC	High performance thin layer chromatography
HPLC	High performance liquid chromatography
RP-HPLC	Reversed phase high performance liquid chromatography
UHPLC	Ultra high performance liquid chromatography
GC	Gas chromatography
LC	Liquid chromatography
SIAM	Stability indicating assay method
ICH	International conference on harmonization
IP	Indian pharmacopeia
USP	United state pharmacopeia
BP	British pharmacopeia

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Conflict of Interest

The Authors do not report any conflict of Interest.

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