QbD APPROACH TO HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FLUOXETINE HYDROCHLORIDE AND OLANZAPINE IN PHARMACEUTICAL DOSAGE FORM

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Abstract: A Quality-by-Design approach to method development can potentially leads to a more robust/rugged method due to emphasis on risk assessment and management than traditional or conventional approach. In QbD approach, the impact and the interaction between critical method variables are understood using a design of experiment approach, which incorporates statistical multi-variate analysis and modeling. This study applied a QbD approach to method development and validation of fluoxetine hydrochloride and olanzapine in combination. An attempt was made to develop validate accurate and précised analytical method. An efficient experimental design based on central composite design of 2 key component of HPLC method (mobile phase and pH) at 3 different level. The chromatographic condition were optimized, i.e; column C18, mobile phase used were 0.02 M Phosphate Buffer pH-4.2 : Acetonitrile : Triethylamine (50:50:0.03, v/v/v) having buffer pH 3.96, flow rate 1ml/min. The described method was linear at 235nm detection wavelength with 10-50 µg/ml for Fluoxetine hydrochloride and 5-25 µg/ml for Olanzapine. The precision ruggedness and robustness values were also within the prescribed limits (<1% for system precision and <2% for other parameters). The LOD & LOD were 0.056 & 0.17 for FLZ and 0.007 & 0.02 for OLZ. %Recovery was 100.06% found for FLZ and 100.47% for OLZ. Chromatographic peak purity results indicated the absence of co-eluting with main peak of Fluoxetine hydrochloride and Olanzapine. The proposed method can be used for routine analysis of fluoxetine hydrochloride & olanzapine in quality control laboratories.

Index terms- QbD, Quality-by-Design, AQbD, analytical QbD, Fluoxetine hydrochloride, Olanzapine, Design of Experiment approach.

1. INTRODUCTION [1-2]

Quality by design is define as "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.[3]"

Application of the QbD approach in product development is mainly characterized by following principles:

- Designing product and its manufacturing process to meet patient needs with respect to safety and efficacy
- Designing manufacturing process to consistently produce product meeting pre-defined quality criteria
- Understanding impact of input parameters on product quality to adequately build the controls at the critical points in the process

1.1 Objective of QbD

- The main objectives of QbD is to ensure the quality products, for that product & process characteristics important to desired performance must be resulting from a combination of prior knowledge & new estimation during development.
- From this knowledge & data process measurement & desired attributes may be constructed.
- Experimental study would be viewed as positive performance testing of the model ability through Design space.
- Ensures combination of product & process knowledge gained during development.

1.2 Analytical QbD

Analytical sciences are considered an integral part of pharmaceutical development. Analytical method and product development go hand in hand during the entire life cycle of any pharmaceutical product. The traditional approach of analytical method development is quite tedious owing to high degree of variability involved at each stage of method development. In order to eliminate the hiccups encountered during method development, the systematic QbD-based approach has slowly been permeating into the mind-set of analytical scientists. Accordingly, efforts have been made to extend QbD approach to analytical method development, popularly termed as "Analytical QbD (AQbD)."

"AQbD is a science and risk-based paradigm for analytical method development, endeavouring for understanding the predefined objectives to control the Critical Method Variables (CMVs) affecting the Critical Method Attributes (CMAs) to achieve enhanced method performance, high robustness, ruggedness and flexibility for continual improvement."

AQbD helps in development of a robust and cost-effective analytical method which is applicable throughout the lifecycle of the product, to facilitate the regulatory flexibility in analytical method.[4]

The major objective of AQbD has been to identify failure modes and establish robust 'Method Operable Design Region (MODR)', which is also called as 'Design Space', within meaningful system suitability criteria and continuous life cycle management.

Among analytical researchers, to date there is no or negligible experience or exposure with the AQbD approach for analytical methods. As today, pharmaceutical industries have many questions and require a lot more discussions on implementation AQbD and its correlation with other components of pharmaceutical quality systems. Literature survey reveals that many researchers have adopted QbD principles to the development of analytical methods and they are termed analyticalQbD (AQbD).[4]

Table 1.1: Implementation of QbD and AQbD

Stage	Product QbD	Analytical QbD
Stage 1	Define quality Target Product Profile (QTPP)	Define analytical target profile(ATP) • Target analytes selection • Technique selection • Method requirements selection
Stage 2	Critical Quality Attributes	Critical quality attributes
Stage 3	Risk Assessment	Risk assessment method operable Design region
Stage 4	Design Space	
Stage 5	Control Strategy	Control strategy
Stage 6	Life Cycle Management	Life cycle management

2. METHODS AND MATERIALS

Instruments and reference standard

HPLC System, FT-IR (Shimadzu), UV spectrophotometer-Shimadzu-1800, pure sample of Fluoxetine hydrochloride and Olanzapine from Alkem Laboratory Pvt. Ltd, ankleshwar.

3. METHODOLOGY

3.1 Preparation of reference standard solution

The standard stock solution was prepared at concentration 1000µg/ml by dissolving 50mg of Fluoxetine hydrochloride and 1000µg/ml by dissolving 50mg of Olanzapine in 50ml mobile phase.

Then sub-standard stock solution was prepared at concentration 100µg/ml by dilute 2.5ml of fluoxetine hydrochloride and 100µg/ml by dilute 2.5 ml of olanzapine in 25 ml mobile phase.

3.2 Selection of detection iso-absorptive wavelength

50µg/ml solution of Fluoxetine hydrochloride was prepared by diluting 5ml of sub-standard stock solution of Fluoxetine hydrochloride up to 10ml using mobile phase in 10ml volumetric flask and the detection of wavelength was carried out by scanning in the range of 200-400nm.

25µg/ml solution of Olanzapine was prepared by diluting 2.5ml of sub-standard stock solution of olanzapine up to 10ml using mobile phase in 10ml volumetric flask and the detection of wavelength was carried out by scanning in the range of 200-400nm.

3.3 Optimization of mobile phase

The suitable Column, Flow rate, Injection Volume, Detection Wavelength and Diluent were optimized for the equipment during the optimization of RP-HPLC method.

Table 3.1: HPLC operating conditions

Sr. no.	Terms	Condition
1	Instrument	HPLC Shimadzu LC-2010 AHT
2	Column	C-18 Phenomenex (250mm X 4.6 mm, 5 µm)
3	Wavelength	235nm
4	Flow rate	1ml/min
5	Injected volume	20µL
6	Diluent	Mobile phase
6	Diluent	Mobile phase

3.4 HPLC Method Development by QbD Approach

HPLC method development using QbD approach was done by following stages,

Stage 1: Quality Target Product Profile (QTPP)
Stage 2: Determine Critical Quality Attributes (CQAs)
Stage 3: Develop a Design Space and Design of Experiment
Stage 4: Risk assessment
Stage 5: Implement a Control Strategy
Stage 6: Manage Product Lifecycle, including Continual Improvement

Stage 1: Quality Target Product Profile (QTPP)

Selection of Quality Target Product Profile is a potential method for identifying variables which directly effect on the quality. Generally, in liquid chromatographic method there are many QTPP-variables is available in terms of system suitability test.

Examination of potential variables was performed in this defined phase. Here in the HPLC method which was developed for the analysis of antipsychotics the quality target product profile chosen was Retention Time, Peak Asymmetry, respectively.

Stage 2: Determine Critical Quality Attributes (CQAs)

Definition of CQAs states that "It is characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality." In the chromatographic method CQAs were considered as method parameter which are directly affected to the quality target product profile.

Here in HPLC method variables selected were Mobile Phase Ratio, pH of Buffer.

These was a better understanding of the specific levels of control require for critical method parameter to maintain the allowable response range, that is, the critical method attributes.

Stage 3: Develop a Design Space and Design of Experiment

Development of design space and design of experiment was done by following 3 phases.

A. Perform experimental design
B. Factorial design
C. Establishment of design space

A. Perform Experiment Design

After defining the method variable, formal experimental designs such as statistical design of experiments were applied to selected method understanding for obtaining in-depth understanding and perform optimization. Here the DOE based on systemic scouting of two key components of the HPLC method one was mobile phase and second was pH is present. It forms a chromatographic database that will assist with method understanding, optimization, and selection.

The DOE helps to eliminate the need for performing a large number of runs achieves desirable results from a limited number of experiments. Since multivariable interaction of variables and process parameter have been studied, and increased understand of method variability, thus there is greater understanding of the method. There is a better understanding of the specific levels of control required for critical method parameter to maintain the allowable response range, that is a critical method attribute.

The experimentally measured responses were then modeled to determine the design space.

B. Factorial Design

Table 3.2: Coded values for Independent Variables

Name of the factor	Coded value	level		
		-1	0	+1
Mobile Phase Ratio	A	(50:50:0.03)	(55:45:0.03)	(60:40:0.03)
pH	B	3.6	4.2	4.8

Table 3.3: Different batches with their respective composition

No. Exp	Batch code	A. Mobile phase composition	B. pH
1	FO_1	+1	-1
2	FO_2	+1	+1
3	FO_3	-1	+1
4	FO_4	-1	-1
5	FO_5	0	0
6	FO_6	0	0
7	FO_7	0	0
8	FO_8	+1	0
9	FO_9	-1	0
10	FO_{10}	0	-1
11	FO_{11}	0	+1

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 API. The best suited experimental conditions shall be optimized using design expert software.

C. Establishment of Design Space

Design space defines as "The multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality" the allowed deviation of the variables was determined within the design space the proven acceptable ranges. The proven acceptable ranges from robust regions where the deliberate variations in the method parameters do not change the CMA. This ensures that the method does not fail downstream during validation testing. Thus, the risk minimized, and quality is assured. If the modeling experiments do not lead to desire responses, method variables can be adjusted, and new experiments performed. And then verified method was used to perform validation experiments to validate the new developed quality method

Stage 4: Risk assessment

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, analysts, instruments, reagents, days, etc.

Stage 5: Implement a Control Strategy

Control strategy can be implemented after the method validation. But 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 implemented to manage risk and ensure the method delivers the desirable method attributes. Another control strategy for the analysis process will be done by doing some procedural controls and monitoring according to appropriate system suitability and other require quality 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.

Stage 6: Manage Analysis Lifecycle, including Continual Improvement

In the management of analysis lifecycle doing a continual improvement is the best way. Here continual improvement can be implemented to redefine ATP and further it can do by following various way,

- Analysis process can be monitored to make sure consistency in quality.
- Periodic maintenance of HPLC instrument, computers, and regularly updating of software and other related instrument and apparatus can be done within laboratory.
- And, do regular updates with new terminology by time in analysis process.

3.5 Analytical Method Validation

Validation is a documented evidence, which provide 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.

Linearity

The linearity of Fluoxetine hydrochloride and Olanzapine was determined by analyzing 5 independent levels of calibration curve in the concentration range of 10-50 µg/ml for Fluoxetine hydrochloride and 5-25 µg/ml for Olanzapine 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.

Precision

A. Repeatability

Measure Peak Area of solution of Fluoxetine hydrochloride and Olanzapine of 50 µg/ml and 25 µg/ml respectively at 235 nm. 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 Fluoxetine hydrochloride at 20, 30 and 40 µg/ml and Olanzapine at 10, 15 and 20 µg/ml concentrations respectively, three times on same day at interval of 1 hour, simultaneously and %RSD was calculated.

% RSD should be less than 2.

C. Inter-Day Precision

Variation of results amongst day is called inter-day precision. Inter-day precision was determined daily by analyzing Fluoxetine hydrochloride at 20, 30 and 40 µg/ml and Olanzapine at 10, 15 and 20 µg/ml concentrations respectively, for three days and %RSD was calculated.

% RSD should be less 2%.

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level (80%, 100% and 120%) of standard addition. Percentage recovery for Fluoxetine hydrochloride and Olanzapine were found out. Recovery between 98- 102 % justifies the accuracy of the method.

LOD and LOQ

The evaluation of the sensitivity of the analytical method was done by lowest limit of detection and lowest limit of quantitation.
LOD was calculated out by using following Formula:
DL = 3.3σ/S
σ = Standard Deviation of the Response
S = Slope
LOQ was calculated out by using following Formula:
DL = 10σ/S
σ = Standard Deviation of the Response
S = Slope

Robustness

Robustness of the method for Fluoxetine hydrochloride and Olanzapine was determined by subjecting the method to slight change in the method condition, individually, the:

- Pump flow rate,
- Mobile Phase ratio

% RSD was calculated.

System Suitability Studies

The system suitability was evaluated by five replicate analyses of Fluoxetine hydrochloride and Olanzapine. The column efficiency and peak asymmetry, Theoretical Plates were calculated for standard solutions.

Assay

Assay preparation (Marketed Formulation)
Label claim: Fluoxetine hydrochloride: 20 mg and Olanzapine: 10 mg

Preparation for Sample stock solution:

20 tablets (each containing 20 mg Fluoxetine hydrochloride and 10 mg Olanzapine) were weighed and powdered.

The tablet powder equivalent to 10 mg of Fluoxetine hydrochloride and 5 mg Olanzapine was accurately weighed and transferred to a 20 ml volumetric flask, about 10 ml of diluent was added, and the flask was sonicated for 15 minutes. Filter this solution with Whatman filter paper. (Fluoxetine hydrochloride-500 mg/ml, Olanzapine-250mg/ml). The volume was made upto the mark with diluent and mixed well.
Working Standard Preparation: Pipette out 1ml from sample stock solution in 10 ml volumetric flask and then make up the volume upto 10ml with diluent, (Fluoxetine hydrochloride- 50 mg/ml, Olanzapine-25 mg/ml).

4. RESULT AND DISCUSSION

4.1 Selection of detection wavelength

Iso-absorptive point of Fluoxetine Hydrochloride and Olanzapine was found to be 235nm.

4.2 Optimization of mobile phase

The mobile phase optimization was successfully done after many trials shown in the below table.

Table 4.1: Optimization of the Chromatographic Conditions

Sr. no.	Mobile phase	Ratio (v/v)	Remark
1	[0.1% v/v Orthophosphoric acid + Triethylamine] pH-3.5 : Acetonitrile : Methanol 100 : 50 µg/ml	60 : 30 : 10	• Retention time of Fluoxetine HCl was too much (20.20 min) • Peak Tailing and peak broadening were also observed
2	Acetonitrile : Dihydrogen Phosphate pH-3 : Triethylamine 100 : 50 µg/ml	40 : 60 :0.2	• Retention time of Fluoxetine HCl was too much (8.60 min) • Peak tailing and peak broadening were also observed
3	0.02 M Phosphate Buffer pH-4.2 : Acetonitrile : Triethylamine 100 : 50 µg/ml	45 : 55 : 0.03	• Peaks were note separate properly and peak tailing was also observed
4	0.02 M Phosphate Buffer pH-4.2 : Acetonitrile : Triethylamine 100 : 50 µg/ml	50 : 50 : 0.03	• Peak tailing was observed in both drug.
5	0.02 M Phosphate Buffer pH-4.2 : Acetonitrile : Triethylamine 100 : 50 µg/ml	55 : 45 : 0.03	• Peak tailing and peak broadening were observed in Fluoxetine hydrochloride
6	0.02 M Phosphate Buffer pH-4.2 : Acetonitrile : Triethylamine 50 : 25µg/ml	50 : 50 : 0.03	• Slightly peak tailing and broadening observed
7	0.02 M Phosphate Buffer pH-4.2 : Acetonitrile : Triethylamine 50 : 25µg/ml	55 : 45 : 0.03	• Peak was obtained proper

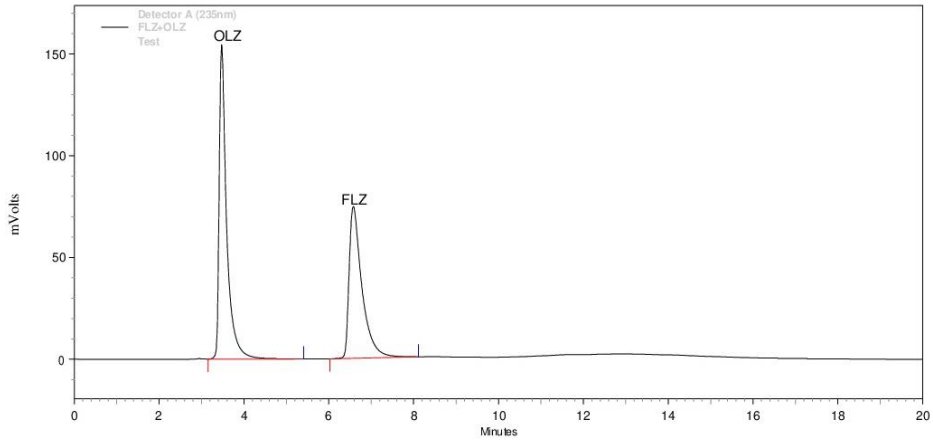

Fig 4.1: Chromatogram in 0.02 M Phosphate Buffer pH-4.2 : Acetonitrile : Triethylamine (55 : 45 : 0.03), (50 : 25µg/ml)

4.3 HPLC Method Development by QbD Approach

Stage 1: Quality Target Product Profile (QTPP)

The Quality Target Product Profile chosen was Retention Time, Area, Peak Asymmetry, Resolution respectively.

Stage 2: Determine Critical Quality Attributes (CQAS)

Critical Quality Attributes selected were Mobile Phase Ratio (0.02 M Phosphate Buffer : Acetonitrile : Triethylamine) and pH of Buffer for HPLC method development.

Stage 3: Develop A Design Space and Design Of Experiment

A. Perform Experimental Design:

Experimental design chosen for HPLC method was Central Composite Design.

Table 4.2 Experiments Model and Condition

File Version	**11.1.2.0**		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Central Composite	**Runs**	11
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	1.0000		

B. Factorial Design:

Table 4.3: Fractional Design Experiment

No. Exp	Batch code	A. Mobile phase Ratio [0.02 M Phosphate Buffer pH-4.2 : Acetonitrile : Triethylamine]	B. pH
1	FO_1	(60:40:0.03)	3.6
2	FO_2	(60:40:0.03)	4.8
3	FO_3	(50:50:0.03)	4.8
4	FO_4	(50:50:0.03)	3.6
5	FO_5	(55:45:.0.03)	4.2
6	FO_6	(55:45:.0.03)	4.2
7	FO_7	(55:45:.0.03)	4.2
8	FO_8	(60:40:0.03)	4.2
9	FO_9	(50:50:0.03)	4.2
10	FO_{10}	(55:45:.0.03)	3.6
11	FO_{11}	(55:45:.0.03)	4.8

Table 4.4: Chromatographic Data Obtain from Experiment Design (CCD)

No. Exp	Batch code	A. Mobile phase Ratio	B. pH	R.T (FLZ & OLZ)	Area	Asymmetry	Theoretical Plates	Resolution
1	FO_1	(60:40:0.03)	3.6	9.233	1746664	1.94	2234.87	11.05
				3.233	2232183	2.27	1801.53	0
2	FO_2	(60:40:0.03)	4.8	10.842	1721334	1.91	1472.49	6.45
				5.442	2333677	2.09	1591.82	0
3	FO_3	(50:50:0.03)	4.8	6.242	1761556	2.24	1820.3	3.53
				4.45	2287855	2.18	1719.23	0
4	FO_4	(50:50:0.03)	3.6	5.375	1782591	2.06	2611.08	6.42
				3.142	22533944	1.89	2093	0
5	FO_5	(55:45:.0.03)	4.2	6.583	1566414	1.8	2656.37	7.83
				3.475	1893938	1.89	2406.15	0
6	FO_6	(55:45:.0.03)	4.2	6.583	1566414	1.8	2656.37	7.83
				3.475	1893938	1.89	2406.15	0
7	FO_7	(55:45:.0.03)	4.2	6.583	1566414	1.8	2656.37	7.83
				3.475	1893938	1.89	2406.15	0
8	FO_8	(60:40:0.03)	4.2	9.792	1753052	1.82	2063.64	9.16
				4.1	2269760	1.97	1851.87	0
9	FO_9	(50:50:0.03)	4.2	5.525	1568104	2.01	2841.74	5.75
				3.517	2033297	1.84	2449.15	0
10	FO_{10}	(55:45:.0.03)	3.6	6.742	1762855	2.07	2376.71	8.72
				3.183	2245811	2.13	2350.09	0
11	FO_{11}	(55:45:.0.03)	4.8	7.817	1766839	2.19	1624.59	5

				4.725	2310995	2.06	1686.14	0

Table 4.4: Evaluated Data for Design Expert Software

		Factor 1	Factor 2	Response 1	Response 2	Response 3
Std	**Run**	**A: mobile phase ratio (ml)**	**B: pH of buffer**	**Retention time of FLZ (min)**	**peak Asymmetry**	**Theoretical Plates**
1	1	50	3.6	5.375	2.06	2611.08
6	2	60	4.2	9.792	1.82	2063.64
4	3	60	4.8	10.842	1.91	1472.49
9	4	55	4.2	6.583	1.8	2656.37
7	5	55	3.6	6.742	2.07	2376.71
8	6	55	4.8	7.817	2.19	1624.59
10	7	55	4.2	6.583	1.8	2656.37
5	8	50	4.2	5.525	2.01	2841.74
3	9	50	4.8	6.242	2.24	1820.3
2	10	60	3.6	9.233	1.94	2234.87
11	11	55	4.2	6.583	1.8	2656.37

B. **Establishment of Design Space: Optimization of various parameters for analysis of Fluoxetine hydrochloride using HPLC (by central composite design)**

Table 4.5 Design Summary for optimization

File Version	**11.1.2.0**		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Central Composite	**Runs**	11
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	1.0000		

Factor	Name	Units	Type	Minimum	Maximum
code				**0**	**1**
A	mL of Acetonitrile	mL	Numeric	50.00	60.00
B	pH of buffer	-	Numeric	3.60	4.80

Table 4.6: Evaluation degrees of freedom of design for optimization of analysis of Fluoxetine Hydrochloride by HPLC

Response	Name	Unit	Analysis	Minimum	Maximum	Ratio	Model
R1	Retention time of FLZ	Min	Polynomial	5.375	10.842	2.02	Quadratic
R2	Peak Asymmetry	-	Polynomial	1.8	2.24	1.24	Quadratic
R3	Theoretical Plates	-	Polynomial	1472.49	2841.74	1.93	Quadratic

PREDICTION OF OPTIMIZED FORMULATION

Table 4.7: Constraints for obtaining optimized formulation Name

Name	Goal	Lower Limit	Upper Limit
A: ml of ACN	Is in range	50	60
B: pH of Buffer	Is in range	3.6	4.8
Retention time of FLZ	Minimize	5.3	5.8
peak Asymmetry	Is in range	1.5	2
Theoretical Plates	Maximize	2000	2841.74

Table 4.8: Obtained solution for optimized formulation

No.	ml of CAN	pH of buffer	Retention time of FLZ	peak Asymmetry	Theoretical Plates	Desirability
1	50.000	3.940	5.313	1.955	2848.068	0.987

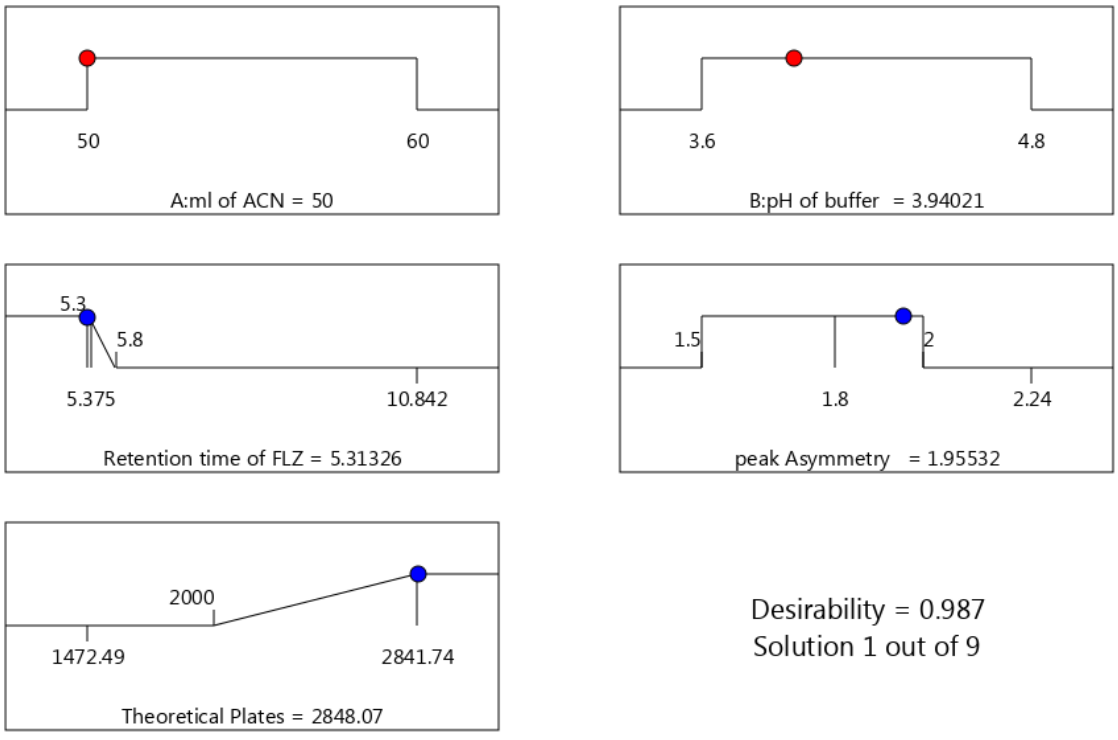

Fig. 4.2: Solution Ramp Accoriding to Inndividule Responses (MODR)

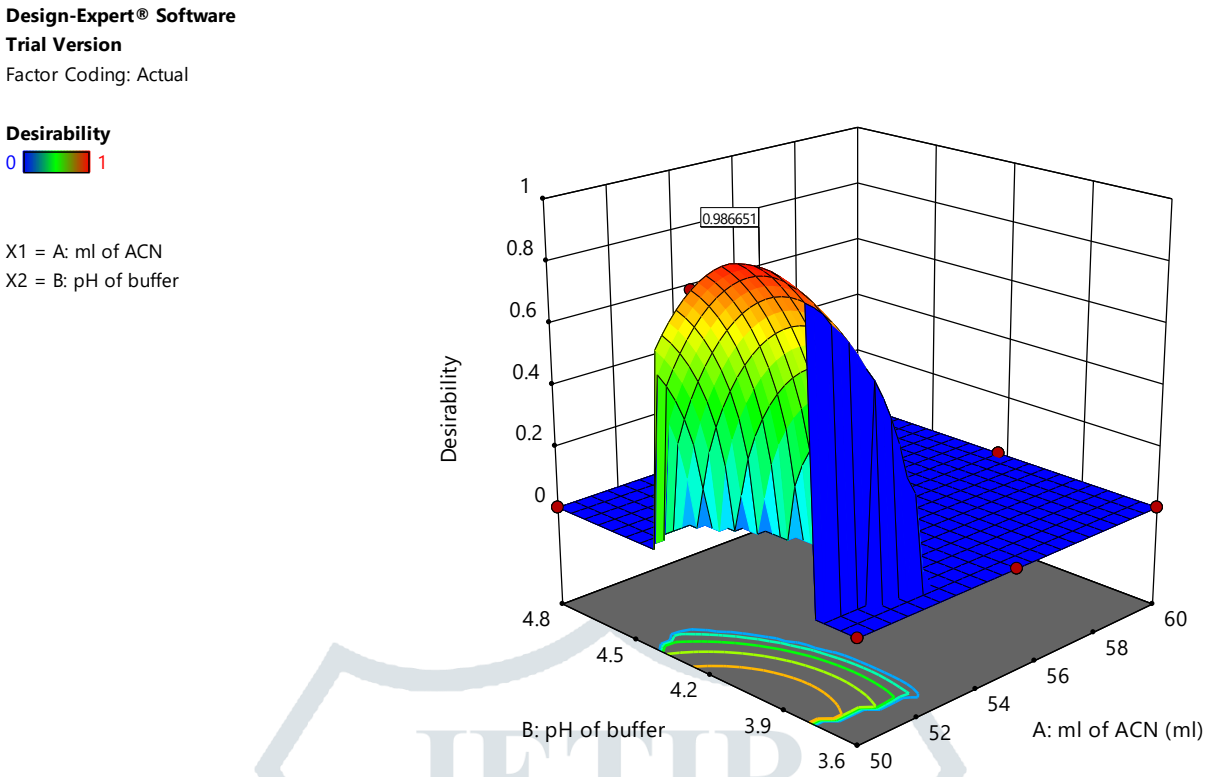

Fig. 4.3: 3D surface plot of Desirability for Obtaining Optimized Formulation

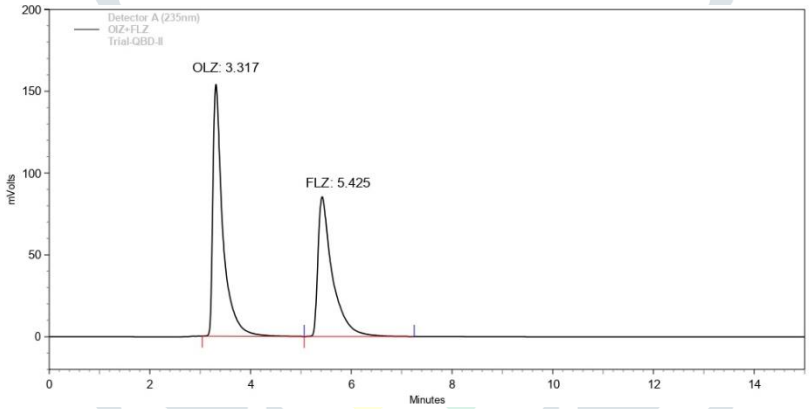

Fig. 4.4: Chromatogram Obtained from the Optimized Formula (0.02 M Phosphate Buffer pH- 3.90 : Acetonitrile : Triethylamine (50 : 50 : 0.03) v/v/v**)**

Table 4.9: Data Comparison after Analysis

Response	Predicted value	Observed value	% predication error
Retation time	5.313	5.425	101.88%
Peak assymetry	1.955	2.19	107.41%
Theoretical plate	2848.068	2328.72	81.73%

Table 4.10: Final Optimized Method Condition

Sr. No	Parameters	Results
1.	Equipment	HPLC Shimadzu LC-2010 AHT
2.	Column	C-18 Phenomenex (250mm X 4.6 mm, 5 µm)
3.	Mobile phase	0.02 M Phosphate Buffer pH- 3.90 : Acetonitrile : Triethylamine (50 : 50 : 0.03) v/v/v
4.	Detected wavelength	235 nm
5.	Injected volume	20µl
6.	Flow rate	1 ml/min
7.	Temperature	30°C

4.4 Method Validation

Linearity :

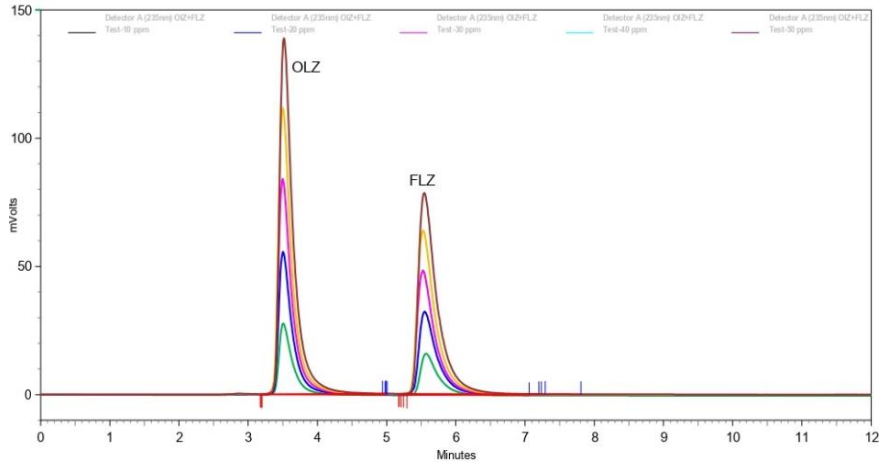

Figure 4.5: Overlain Chromatogram of linearity of Fluoxetine hydrochloride in 10-50 µg/ml and Olanzapine in 5-25 µg/ml

Table 4.11: Linearity for Fluoxetine hydrochloride

Sr. No	Conc(µg/ml)	Peak Area (Mean ± SD) ;(n=5)
1	10	293943
2	20	590874
3	30	883025
4	40	1177073
5	50	1474986

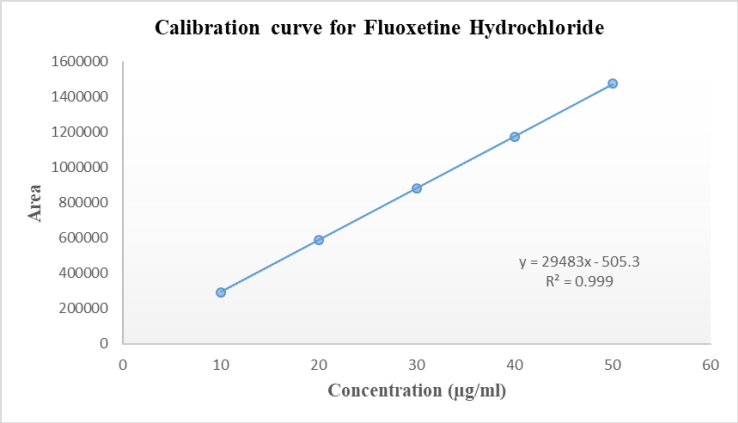

Figure 4.6: Calibration Curve for Fluoxetine hydrochloride (10-50 µg/ml)

Table 4.12: Linearity for Olanzapine

Sr.No	Conc(µg/ml)	Peak Area (Mean ± SD) ;(n=5)
1	5	385147
2	10	768362
3	15	1155580
4	20	1538815
5	25	1924275

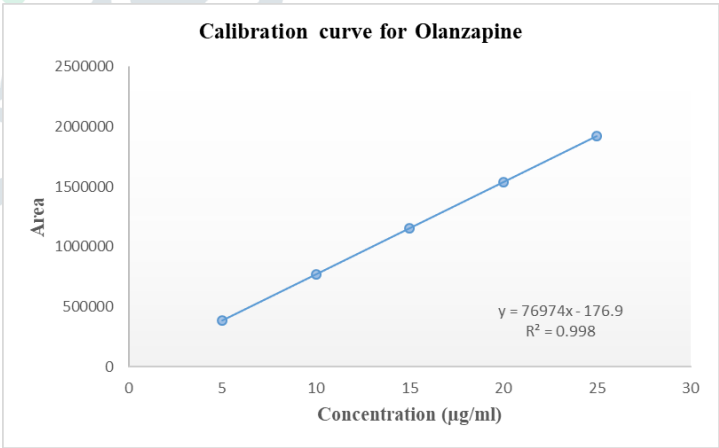

Figure 4.7: Calibration Curve for Olanzapine (5-25 µg/ml)

Repeatability: %RSD for 50 µg/ml Fluoxetine hydrochloride and 25 µg/ml Olanzapine were found to be 0.4790 and 0.3658 for 6 replicates.

Interday Precision and Intraday Precision:

Table 4.13: Precision for Fluoxetine hydrochloride

Sr.No	Precision Period	Con.(µg/ml)	Mean (n=3)	S.D (n=3)	%RSD
1	Interday Precision	20	592272.33	1968.160139	0.3323
		30	885016.33	3934.126629	0.4445
		40	1178318.67	1182.174409	0.1003
2	Intraday Precision	20	593083.00	1914.103707	0.3227
		30	883358.33	1105.360725	0.1251
		40	1176695.67	1945.638541	0.1653

Table 4.14: Precision for Olanzapine

Sr.No	Precision Period	Con.(µg/ml)	Mean (n=3)	S.D (n=3)	%RSD
1	Interday Precision	10	768564	1192.896894	0.1552
		15	1160712	5602.498461	0.4827
		20	1537729.333	2575.268983	0.1675
2	Intraday Precision	10	769163.3333	694.1832131	0.0903
		15	1156444.333	2217.647477	0.1918
		20	1540463.667	1931.625568	0.1254

Accuracy:

Table 4.15: Recovery of Fluoxetine hydrochloride and Olanzapine

Level & Amount from the sample FLZ:OLZ 50mg:25mg	Amount of Standard Spiked (mg)		Peak Area		Total amount Recovered (µg/ml) ± SD (n=3)		% Recoverd of spiked ammount ± SD (n=3)	
	FLZ	OLZ	FLZ	OLZ	FLZ	OLZ	FLZ	OLZ
Blank	-	-	1478318	1922845	-	-	-	-
80%	40	20	2663012	3473470	40.18	20.14	100.46	100.72
100%	50	25	2952387	3854759	50.00	25.10	99.99	100.39
120%	60	30	3242850	4252767	59.85	30.27	99.75	100.90

LOD and LOQ:

Table4.16: LOD and LOQ of Fluoxetine hydrochloride and Olanzapine

Parameters	Results	
	FLZ	OLZ
Standard deviation of the Y-intercepts of the calibration	505.3	176.9
Mean slope of the calibration curves; (n=3) S	29483	76974
LOD (µg/ml)	0.05655	0.00758
LOQ (µg/ml)	0.1713	0.0229

Robustness:

Table 6.17: Robustness for Fluoxetine hydrochloride

Sr.No	Parameter	Mean	SD	%RSD
1	Flow rate -2	1848605	208.16	0.0113
2	Flow rate +2	1220964	360.55	0.0295
3	Buffer -2	1782591	104.00	0.0058
4	Buffer+2	1568104	217.99	0.0139

Table 6.18: Robustness for Olanzapine

Sr.No	Parameter	Mean	SD	%RSD
1	Flow rate -2	2399014	260.70	0.0109
2	Flow rate +2	1601361	62.58	0.0039
3	Buffer -2	22533944	412.85	0.0018
4	Buffer+2	2033297	161.60	0.0079

Assay:

Table 6.19: Assay of Fluoxetine hydrochloride and Olanzapine

Sr. No.		Label claim Mg	Amount found (mg)	Peak area	% Assay	%ASSAY±SD	%RS D of assay
1	FLZ	20	50.38	1484959	100.77	100.27±0.44%	0.43%
2		20	49.99	1473228	99.97		
3		20	50.03	1474583	100.06		
1	OLZ	10	25.02	1925701	100.08	100.20±0.32%	0.32%
2		10	24.92	1917910	99.67		
3		10	25.07	1929897	100.30		

5. CONCLUSION

Fluoxetine hydrochloride (an anti-depressant) and Olanzapine (An Anti-psychotic) is a novel compound use to treat affective disease. The combination of these two drugs is more effective at reducing the symptoms of treatment-resistance depression with psychotic feature either than drug when used as monotherapy.

A Quality-by-Design approach to HPLC method development has been described. The method goals are clarified based on the process understanding. The experimental design describes the scouting of the key HPLC method components including mobile phase and pH. Their interrelationships are studied and optimized. QbD principles were applied to HPLC method development for Fluoxetine hydrochloride and olanzapine using Design Expert Software by CCD. And a multivariant analysis of several critical method parameters like combination of 2 factor at 3 different level was done used to determine the best performing chemistry system and the final Design space. 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.

QbD Approach to method development has helped to better understand the method variables, leading less chance of failure during method validation and transfer. The automated QbD method Development Approach using Design Expert software has provided a better performing more robust method in less time compared to manual method development.

The chromatographic condition were optimized, i.e; column C18, mobile phase used were 0.02 M Phosphate Buffer pH-4.2 : Acetonitrile : Triethylamine (50:50:0.03, v/v/v) having buffer pH 3.96, flow rate 1ml/min. The described method was linear at 235nm detection wavelength with 10-50 µg/ml for Fluoxetine hydrochloride and 5-25 µg/ml for Olanzapine. And the observed result was mention in beloved table,

PARAMETER	**RESULT**	
	Fluoxetine Hydrochloride	**Olanzapine**
Linearity and Range	10-50 (µg/ml)	5-25 (µg/ml)
Correlation Coefficient R^2	0.999	0.998
Regression Equation	Y=29483x-505.3	Y=76974x-176.9
Repeatability (%RSD)	0.071	0.054
Intra-Day Precision (%RSD)	0.32-0.16	0.09-0.12
Inter-Day Precision (%RSD)	0.33-0.10	0.15-0.16
Accuracy	100.06%	100.47%
LOD (µg/ml)	0.056	0.007
LOQ (µg/ml)	0.1713	0.0229
Robustness %	0.015	0.006
Assay (n=3)	100.27 ± 0.44%	100.2 ± 0.32%

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