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Application of Combined Mixture Design-DoE in Quality Improvement and Robustness Testing of Related Substances Method for Quetiapine Fumarate by RP-HPLC

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Abstract:

The present study aims to apply the Design of Experiments (DoE), to develop quality improvement and robustness testing method for related Substance of Quetiapine fumarate by RP-HPLC. In this study, the DoE Combined- Randomized method was used. The drug was analyzed on Zorbax Eclipse Plus C8 Column (250×4.6mm, 5µ) using UV Detector. The Mobile phase consisting of Di-ammonium Hydrogen Phosphate (0.02 M), methanol, and acetonitrile with a flow rate of 1.3 ml/min. In DoE, evaluated variables are Solvent composition and salt concentration. The detection wavelength was 230 nm. The Design of Experiments allows interpreting the results with better outcome and enhances understanding. Conclusively DoE as an efficient tool for determining Related Substances of Quetiapine fumarate and Design is validated. The proposed method was successfully demonstrated as a Quality improvement, robustness testing method which can be used as another method for the analysis of the related substance of Quetiapine fumarate in routine analysis.

Keywords: Design of Experiments (DoE), OFAT, Related Substances, RP-HPLC, Quetiapine Fumarate

INTRODUCTION:

Quetiapine fumarate is a dibenzothiazipine derivative and is chemically 2- [2-(4-Dibenzo [b, f] [1, 4] thiazepin-11-yl-piperazinyl) ethoxy] ethanol fumarate having Molecular formula $C_{42}H_{54}N_6O_8S_2$ and Molecular weight 883.1 gm/mol [1]. It is an oral antipsychotic drug act as an antagonist of multiple neurotransmitters, including Serotonin and Norepinephrine. It is a Selective monoaminergic antagonist with the high affinity of Serotonin type 2 ($5HT_2$) and Dopamine Type 2 (D_2) receptors.[1] It is prescribed in the treatment of Schizophrenia or maniac episodes associates with bipolar disorder and renal impairment. This drug has rapid absorption with peak plasma concentration attained in 1.5 hrs. Its half-life is approximately 6 hrs. CYP_3 A_4 metabolizes the drug. The chemical structure of Quetiapine fumarate [1] was depicted in figure 1.

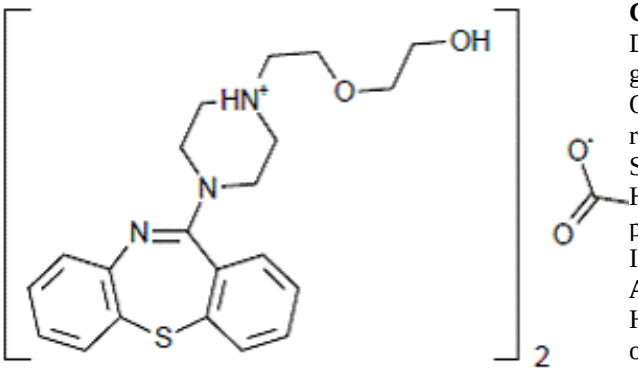

Figure 1: Chemical structure of Quetiapine fumarate

Literature survey revealed that few analytical methods have been reported for estimation of Quetiapine fumarate in bulk and its pharmaceutical formulations. The reported methods include Stability Indicating RP-HPLC [2-4], RP-HPLC [5-9], DoE in the bioanalytical determination of Quetiapine fumarate in human plasma by RP-HPLC [10], Bio-analytical RP- HPLC [11], there are no reports as per our knowledge that methods developed for the analysis of Related Substances for Quetiapine fumarate by DoE. So, the present study was aimed to develop quality improvement and robustness testing method of Related Substances for Quetiapine fumarate by RP-HPLC. To achieve the aim of the present study, the application of the combined mixture design of DoE has been embraced for good resolution and Selectivity of impurities [12-15].

MATERIAL AND METHODS:

Chemicals and Reagents:

Dihydrogen ammonium phosphate analytical reagent grade (Merck), Quetiapine System Suitability (USP), Quetiapine related compound G (USP), Quetiapine related compound B (USP), Quetiapine related compound S-Oxide, Quetiapine related compound N- Oxide (USP). HPLC grade Methanol and HPLC grade acetonitrile purchased from Merck life sciences Pvt Ltd, Mumbai, India. Milli-Q water system was used for graded water.

Apparatus and Equipment:

HPLC System (Waters HPLC, Alliance make Consisting of Zorbax Eclipse Plus C8 Column (250×4.6 mm, 5µ) and UV Detector was employed. Chromatographic data were acquired using Empower 3 Software. Micro-Balance,Digital electronic analytical balance (Sartorius),

Sonicator (Soltec). Design Expert 10 ® Software was used in DoE studies.

One Factor at A Time (OFAT) Approach:

Different experiments with the OFAT approach are conducted as per the USP Method [16-17]. In the USP method, Impurity S-oxide was not listed, and the resolution between the Impurity B & Quetiapine Fumarate is very close. By using the Design of Experiments, all the listed impurities, as stated in USP along with S-oxide, were targeted for good resolution and Selectivity.

Preparation of mobile phase solutions:

Accurately weighed quantity (5.2 g ,3.9615 g and 6.6025 g) of Dihydrogen ammonium phosphate was dissolved and diluted up to 2 liters with milli-Q Water, filtered through 0.45µ filter paper and sonicated for 5 min to obtained 0.02M, 0.015 M and0.025 M buffer solution. Water and acetonitrile (50:50 v/v) were used as the diluent. Mobile phase A is Dihydrogen ammonium phosphate buffer; Mobile phase B is Methanol; Mobile phase C is Acetonitrile was taken an appropriate concentration suggested by DoE.

Preparation of Quetiapine Fumarate System Suitability Solution:

Accurately weighed 2.5 mg quantity of drug was transferred, dissolved and made up to 5 ml with buffer solution, sonicated for 5min.

Preparation of Stock and working standard solutions:

Weighed quantity 2.5 mg each of impurities G, B, S-Oxide, N-Oxide of Quetiapine fumarate was dissolved and diluted up to 25 ml with buffer solution and sonicated for 5 min. Impurities G, S-Oxide mixture and Impurity B, N-Oxide mixture solutions were prepared by pipetted out 2 ml of Quetiapine related compound G stock solution, and 4 ml of Quetiapine related compound S-Oxide stock solution were transferred in a 100ml volumetric flask and made up to 100ml with buffer. Impurity B & N-Oxide Mixture solution was prepared by pipetted out 2 ml of Quetiapine related compound B stock solution, and 6 ml of Quetiapine related compound N-Oxide stock solution was transferred in a 100ml volumetric flask and made up it with buffer.

Preparation of Quetiapine fumarate and all Impurities mixture solution:

Accurately weighed quantity 18 mg of Quetiapine fumarate (USP) was transferred in to a 100 ml volumetric flask and dissolved in 30 ml buffer solution and to this added 2 ml, 4 ml of Quetiapine related compound G and Quetiapine related compound S-Oxide stock solution and 2 ml, 6ml of Quetiapine related compound B and Quetiapine related compound N-Oxide stock solutions were transferred and made up to 100 ml with same buffer solution.

Application of DoE during the method development:

The design of experiments considers multiple factors to experiment in a single experiment, and all the factors were varied in each of the sets of experiments as per predetermined statistical modelling. A simple combined-randomized design was optimized to develop the method for the related substances for Quetiapine Fumarate with each high and low levels of each selected factor or variable. Four different factors or variables are selected to determine the lack of fit or curvature of the design. A total of 28 runs of different combinations are given by the DoE which differ in the mobile phase composition, Salt concentrations, and the trails are executed using HPLC.

Design Prediction and Validation:

Evaluation of further results for the effects of variables on responses with the help of Trace plots, Contour plots, mix process plots, 3D Mix process plots to understand which variable has a significant effect on the responses. Further, Prediction of solutions as per the desired outcome and validating suggested solutions against experimental data. Two solutions were selected from DoE Predicted solutions and evaluate with numerical optimization and overlay graph to understand the method operable design region of experimental Design.

RESULTS & DISCUSSION:

The constraints or variables are selected were shown in Table 1.

Table 1: DoE Design Summary

Study Type	Combined type
Subtype	Randomized type
Design Type	I-optimal type
Design Model	Reduced Quadratic x Quadratic Model
Runs	28 runs
Blocks	No Blocks

Table 2: ANOVA Evaluation

S. No.	R1	R2	R3	R4	R5	R6	R7
R-Squared	0.9988	0.7787	0.7242	0.8798	0.9184	0.9363	0.6196
Adjusted R-Square	0.9984	0.7260	0.6741	0.8264	0.8586	0.8895	0.5879
Predicted R-Square	0.9974	0.6288	0.4857	0.6893	0.5833	0.6416	0.5492
Adequate Precision	150.077	11.362	13.610	15.554	16.163	14.729	12.682

R1: Retention Time of Quetiapine Fumarate
R2: Resolution between Fumaric acid and S- Oxide
R3: Resolution between S-Oxide & N-Oxide
R4: Resolution between N-Oxide & Impurity G
R5: Resolution between Impurity G & Impurity B
R6: Resolution between Impurity B & Quetiapine Fumarate
R7: Resolution between Impurity G & Quetiapine Fumarate

ANOVA Evaluation

ANOVA indicates the statistical significance of the model. The adjusted R square and predicted R square values should be in reasonable agreement (difference less than 0.2), and adequate precision shall be more than 4, which indicated that the model was capable in predicting solutions from the data available from Experimental runs and that there is a good correlation b/w studies variable and observed responses. The ANOVA evaluation results were depicted in Table 2.

The fraction of Design Space Evaluation:

FDS discuss the design space that is being predicted by Design. Ideal FDS score is 80% or 0.8 or above and 100% for the Quality by Design work. In the present Design, the obtained FDS score from the graph is found to be 0.93, which is in the range to accept the Design. So, Design can be used further to obtain the best results. The result of the fraction of the design space was shown in Figure 2.

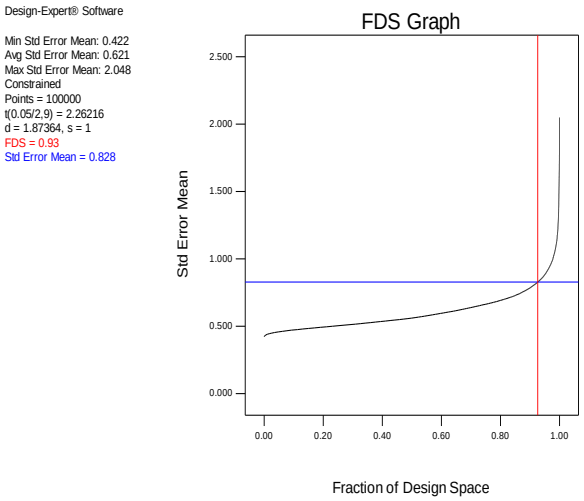

Figure 2: Fraction of Design Space

Table 3: Experimental Setup by DoE

Runs	A#	B#	C#	D#	R1*	R2*	R3*	R4*	R5*	R6*	R7*
	%	%	%	M							
1	42.74	52.25	5	0.015	18.094	11.45	3.69	1.79	12.23	0.76	8.11
2	37.11	52.88	10	0.02	9.633	5.66	2.36	1.37	8	-0.26	4.01
3	41.16	50	8.83	0.025	15.526	9.48	2.72	2.04	9.7	1.11	6.85
4	42.58	50	7.416	0.02	17.167	12.82	2.98	1.86	11.29	1.19	7.07
5	36.41	56.17	7.40	0.015	9.413	9.66	2.61	1.2	8.24	-1.41	6.11
6	39.85	55.14	5	0.015	13.561	9.3	3.36	1.91	11.52	0.02	6
7	38.36	54.63	6.99	0.025	12.19	9	2.89	1.53	8.14	0.36	5.2
8	42.58	50	7.41	0.02	17.16	12.82	2.98	1.86	11.29	1.19	7.07
9	33.02	56.97	10	0.015	7.00	8.21	2.78	1.19	5.87	-1.25	4.12
10	35.80	59.12	5.07	0.025	9.76	8.16	2.92	1.13	6.66	-0.13	4.12
11	42.38	50	7.61	0.015	16.71	10.55	2.69	1.83	15.32	0.81	7.85
12	36.41	56.17	7.40	0.015	9.41	9.66	2.61	1.2	8.24	-1.41	6.11
13	36.88	53.11	10	0.015	9.47	8.03	2.18	1.18	8.15	-1.14	5.81
14	36.33	56.03	7.63	0.02	9.33	8.65	2.51	1.25	8.78	-0.23	2.71
15	34.80	60	5.19	0.015	8.33	8.8	3.07	1.02	8.43	-1.48	4.89
16	36.33	56.03	7.63	0.02	9.33	8.65	2.51	1.25	8.78	-0.23	2.71
17	30.09	60	9.90	0.015	5.67	5.89	1.87	0.85	7.62	-1.77	3.04
18	38.36	54.63	6.99	0.025	12.19	9	2.89	1.53	7.17	0.87	5.2
19	41.89	53.10	5	0.025	18.18	9.96	2.49	1.39	8.52	1.43	7.94
20	31.05	60	8.94	0.025	6.38	6.76	-0.62	2.36	4.6	1.01	4.22
21	30	60	10	0.02	5.68	7.19	2.28	0.88	6.39	-1.5	3.54
22	34.79	55.20	10	0.025	8.41	4.66	-0.77	2.86	5.36	0.82	3.49
23	40	50	10	0.02	12.60	9.91	3.09	2.27	9.31	0.34	5.26
24	39.71	55.28	5	0.02	13.16	11.25	3.57	1.73	10.03	0.36	5.6
25	36.33	56.03	7.63	0.02	9.33	8.65	2.51	1.25	8.78	-0.23	2.71
26	34.49	60	5.50	0.02	8.06	8.52	2.68	1.04	7.97	-1.03	5.7
27	45	50	5	0.025	26.09	12.99	-4.23	8.79	12.31	3.06	11.7
28	42.61	52.38	5	0.02	17.80	12.46	3.66	1.92	11.35	1.23	7.53

#A: Buffer, B: Methanol, C: Acetonitrile, D: Salt Concentration, M: Molarity
**R1: Retention Time of Quetiapine Fumarate*
R2: Resolution between Fumaric acid and S- Oxide.
R3: Resolution between S-Oxide & N-Oxide
R4: Resolution between N-Oxide & Impurity G
R5: Resolution between Impurity G & Impurity B
R6: Resolution between Impurity B & Quetiapine Fumarate
R7: Resolution between Impurity G &Quetiapine Fumarate

Table 3: Constraint Selection

Names	Goals	Lower Limits	Upper Limits	Lower Weights	Upper Weights	Importance
#A	Within range	30	45	1	1	3
#B	Within range	50	60	1	1	3
#C	Within range	5	10	1	1	3
#D	Within range	0.015	0.025	1	1	3
*R1	none	5.672	18.18	1	1	3
*R2	None	2	12.82	1	1	3
*R3	Is in Range	1.5	3.69	1	1	3
*R4	Is in Range	2	2.86	1	1	3
*R5	None	4.6	15.32	1	1	3
*R6	None	-1.77	1.43	1	1	3
*R7	Is in Range	2.71	8.11	1	1	3

#A: Buffer, B: Methanol, C: Acetonitrile, D: Salt Concentration.
**R1: Retention Time of Quetiapine fumarate*
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R4: Resolution between N-Oxide & Impurity G
R5: Resolution between Impurity G & Impurity B
R6: Resolution between Impurity B & Quetiapine fumarate
R7: Resolution between Impurity G & Quetiapine fumarate

Table 4: Design predicted solutions

S. No	# A	#B	#C	# D	*R1	*R2	*R3	*R4	*R5	*R6	*R7	
1	44.2	50.0	5.7	0.02	20.4	13.2	3.69	2.0	12.2	1.8	7.68	Selected
2	44.2	50.3	5.3	0.02	20.5	13.0	3.67	2.0	12.2	1.8	7.66	
3	44.3	50.0	5.6	0.02	20.5	13.3	3.68	2.1	13.1	1.8	7.68	
4	44.3	50.0	5.6	0.02	20.5	13.2	3.67	2.0	12.9	1.8	7.67	
5	44.2	50.0	5.7	0.02	20.4	13.2	3.62	2.0	12.2	1.8	7.62	
6	44.3	50.0	5.6	0.02	20.5	13.2	3.68	2.1	13.0	1.8	7.67	
7	44.2	50.0	5.7	0.02	20.4	13.2	3.65	2.0	12.6	1.8	7.64	
8	44.3	50.0	5.6	0.02	20.5	13.2	3.67	2.0	12.9	1.8	7.66	
9	44.2	50.0	5.7	0.02	20.4	13.2	3.63	2.0	12.4	1.8	7.62	
10	44.3	50.0	5.6	0.02	20.5	13.3	3.68	2.1	13.0	1.8	7.68	
11	44.2	50.0	5.7	0.02	20.4	13.2	3.64	2.0	12.5	1.8	7.63	
12	44.2	50.0	5.7	0.02	20.4	13.2	3.64	2.0	12.5	1.8	7.64	
13	44.3	50.0	5.6	0.02	20.5	13.3	3.68	2.1	13.0	1.8	7.67	
14	44.3	50.0	5.6	0.02	20.5	13.2	3.67	2.0	12.8	1.8	7.66	
15	44.2	50.0	5.7	0.02	20.4	13.2	3.63	2.0	12.4	1.8	7.63	
16	44.2	50.0	5.7	0.02	20.4	13.2	3.62	2.0	12.3	1.8	7.62	
17	44.3	50.0	5.7	0.02	20.5	13.2	3.66	2.0	12.7	1.8	7.65	
18	44.2	50.0	5.6	0.02	20.5	13.2	3.66	2.0	12.6	1.8	7.66	
19	44.3	50.0	5.6	0.02	20.5	13.2	3.66	2.0	12.8	1.8	7.66	
20	44.3	50.0	5.6	0.01	20.4	13.2	3.69	2.1	13.4	1.7	7.66	
21	44.2	50.0	5.6	0.01	20.3	13.2	3.69	2.1	13.5	1.7	7.65	
22	44.2	50.0	5.7	0.01	20.2	13.2	3.69	2.1	13.7	1.7	7.64	
23	43.9	50.9	5.1	0.02	20.0	12.7	3.69	2.0	12.0	1.6	7.61	Selected
24	43.8	51.1	5.0	0.02	19.9	12.5	3.69	2.0	12.0	1.6	7.59	
25	43.8	51.1	5.0	0.02	19.9	12.5	3.69	2.0	12.0	1.6	7.59	
26	44.2	50.0	5.7	0.01	20.1	13.2	3.69	2.1	14.3	1.6	7.62	
27	43.7	51.2	5.0	0.02	19.7	12.5	3.68	2.0	12.0	1.6	7.57	
28	44.3	50.0	5.6	0.01	20.7	13.3	3.68	2.0	16.0	1.6	7.69	

#A: Buffer, B: Methanol, C: Acetonitrile, D: Salt Concentration,
**R1: Retention Time of Quetiapine Fumarate*
**R2: Resolution between Fumaric acid and S- Oxide.*
**R3: Resolution between S-Oxide & N-Oxide.*
**R4: Resolution between N-Oxide & Impurity G*
**R5: Resolution between Impurity G & Impurity B*
**R6: Resolution between Impurity B & Quetiapine fumarate*
**R7: Resolution between Impurity G & Quetiapine fumarate.*

Table 5: Observations for Mix Process Plots

Responses	A	B	C	D
R1	+ve	+ve	+ve	-
R2	+ve	+ve	+ve	-
R3	+ve	+ve	+ve	-ve
R4	+ve	+ve	-ve	+ve
R5	+ve	+ve	+ve	+ve
R6	+ve	+ve	+ve	+ve
R7	+ve	+ve	+ve	-

+, Positive effect. -, Negative effect, -, No effect
A: Buffer, B: Methanol, C: Acetonitrile, D: Salt Concentration,
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R4: Resolution between N-Oxide & Impurity G
R5: Resolution between Impurity G & Impurity B
R6: Resolution between Impurity B & Quetiapine fumarate
R7: Resolution between Impurity G &Quetiapine fumarate.

Factors and Variables Selection:
Mobile phase composition is taken as variable and salt concentration is taken as a factor shown in Table 3.

Design Evaluation:
Statistical measures of power, lack of fit, pure error are the three parameters that determine the adequacy of the model created. Those are evaluated to ensure the design adequacy. Experimental runs are executed, and results were evaluated for statistical significance by ANOVA to prove the adequacy. Statistical measures like Model F Value, Adjusted R-Square, Predicted R square, adequate precision an included in ANOVA.

Graphical Evaluation:
Graphical evaluation is done by evaluating the perturbation plot, which helps in comparing the all factors at a single point in a design. Contour-Plot is a 2-dimensional plot (2D) after responses that are plotted with the combination of factor numeric/Mixture component, 3D Surface Plots projects the contour plot, and Model graphs. Graphical evaluation data was shown in figure 3, figure 4 and figure 5 and figure 6.

Design Validation with Selected and Predicted Solutions:
Finalized Solutions was optimized by the Design; in this design, DoE predicted 28 types of solutions with variations in each factor and variable in each run. Design validation, Predicted Solution data was shown in Table 3, 4 and 5.and Optimized chromatograms of solution 1 and solution 23 were depicted in figure 7 and figure 8.

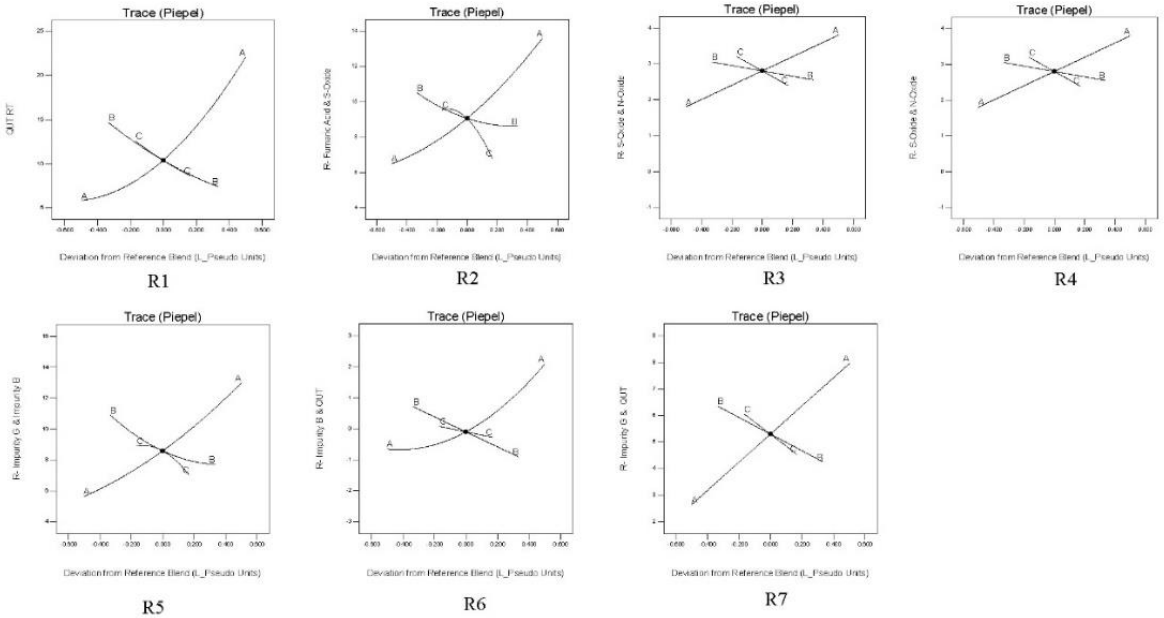

Figure 3: Trace Plots R-1 to R-7

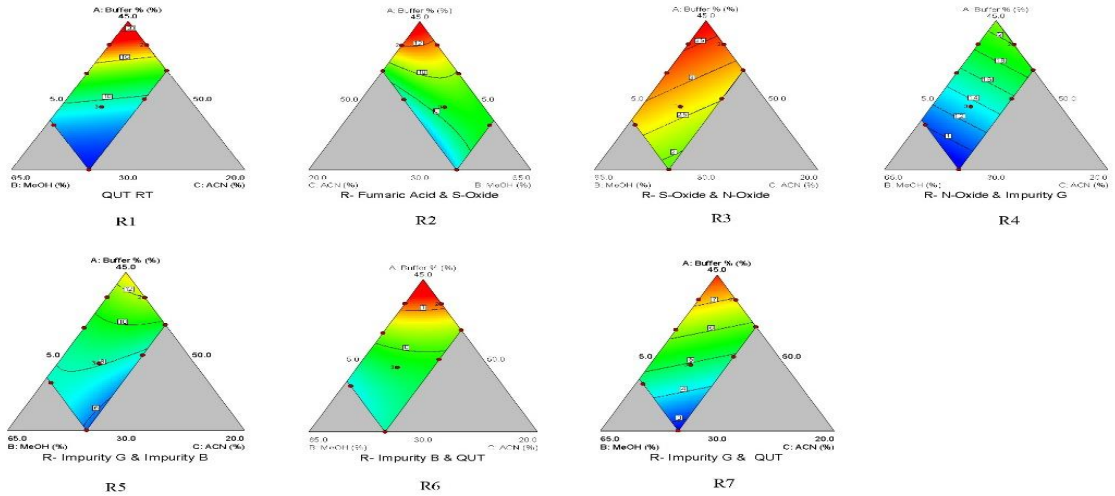

Figure 4: Contour Plots R-1 to R-7

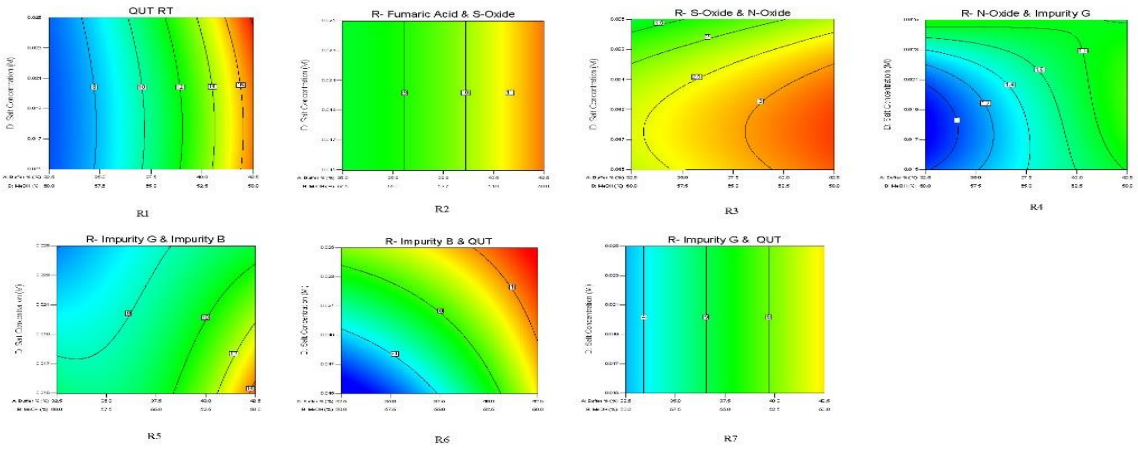

Figure 5: Mix Process Plots R-1 to R-7

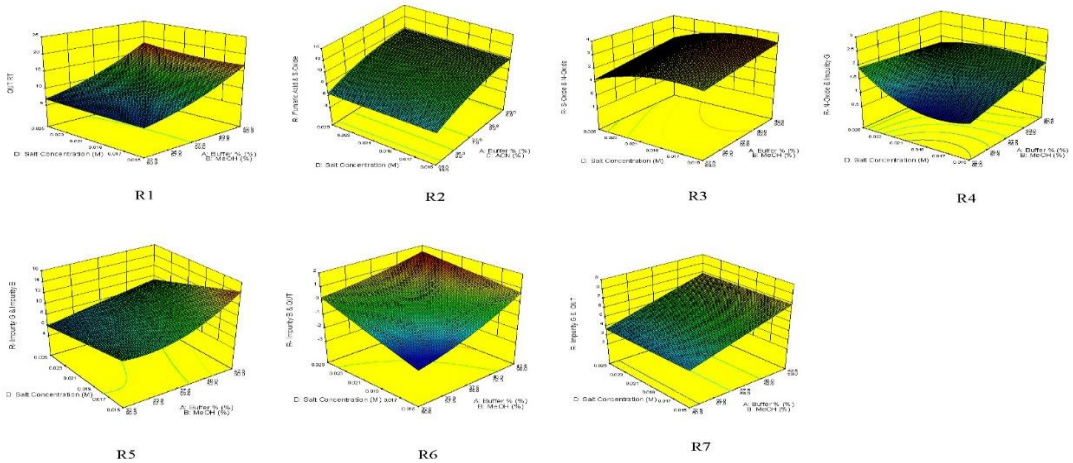

Figure 6: 3D Mix Process Plots R-1 to R-7

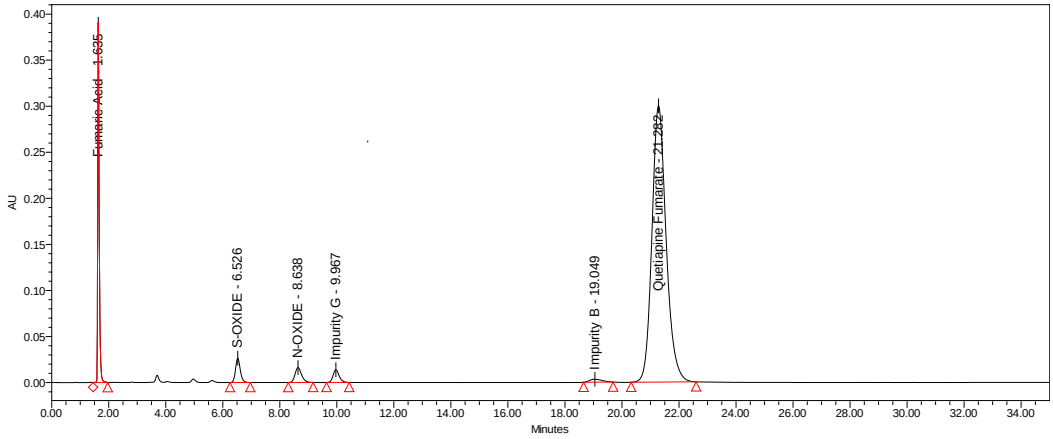

Figure 7: Quetiapine and all impurities Chromatograms of Solution 1

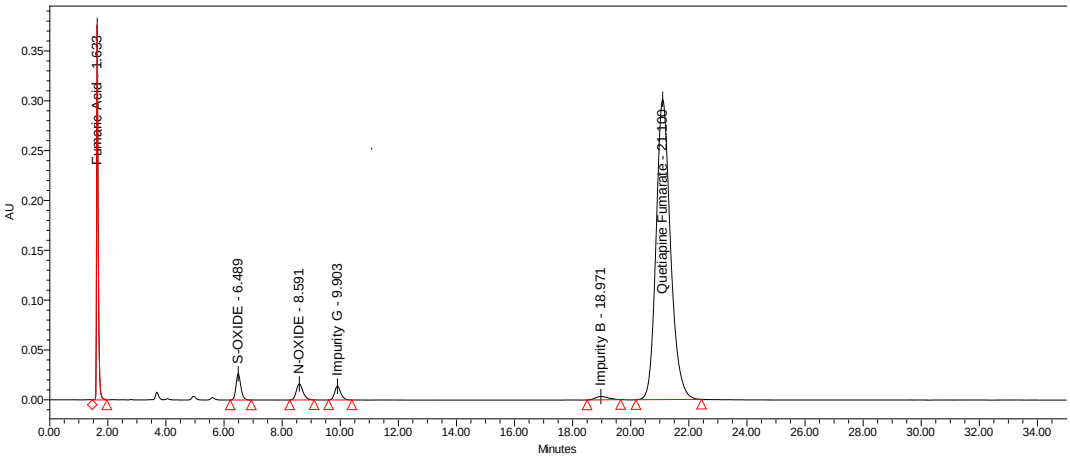

Figure 8: Quetiapine and all impurities Chromatograms of Solution 23

CONCLUSION:

In the present study, Quetiapine fumarate is selected as a suitable product to evaluate the application of DoE in Related substance method development. Related substance profile studies are crucial for drug development to compare the stability of drug products and to prove the stability-indicating nature of the applied test method. A similar substance method of Quetiapine fumarate has very critical factors like to separate Impurity S-Oxide, which was not listed in the USP method, and to increase the resolution between Impurity B & Quetiapine fumarate. The model has given predicted solutions of 28 different combinations of selected factors along with the anticipated results. The desirability for the given solution has a significant role. Desirability factor 1 solution will provide better resolution than the other with less than 1 out of the predicted solution; solution-1 and solution-23 were selected and experimented with deriving practical results with the given combination of factors. The practical results of the Related Substance profile are closely matching with that of predicted solutions.

With the current scope of study DoE as an effective tool for Related Substance method development with multiple impurities that are having less runtime and can be used for RS, methods are employed and validated to prove its efficacy. The developed method can be further utilized for routine analysis.

ACKNOWLEDGEMENT:

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CONFLICT OF INTEREST:

All authors report no conflict of interest directly or indirectly in the publication of this manuscript.

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