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Application Of Quality By Design Approach For Development & Validation Of Rp-Hplc Method For Lumateperone Tosylate Drug In Bulk & It's Capsule Dosage Form

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The goal of the current investigation is to use the Quality by Design approach to grow an exact, specific, time saving and work saving RP-HPLC method for the establishment of lumateperone tosylate in bulk and its capsule form. Quality by Design (QbD) refers to the achievement of certain expected quality with proper and pre-set specifications. An important part of the QbD is the grasp of dependent variables, various factors, and their interaction effects by a desired set of trials on the responses to be analyzed. QbD is a first prospective approach through which, before starting, know the process, what parameters hamper it, and build quality throughout the process, not at last. Using ATPP, CPP, DoE, CQA, and Design Space. The given method was developed using the QbD approach. Given the developed method, separation was done with a column Phenomenex C18 (250 mm x 4.6 mm ID, particle size 5 µm). at a flow rate of 1.00 ml/min using the mobile phase Methanol:0.1% OPA in water (45:55% V/V) at a wavelength of 227 nm with a chromatographic run time of 10 min. The method was linear over the range of 2-10 µg/ml with a correlation coefficient of 0.9999 for Lumateperone tosylate. The method was validated by using the ICH Q2 (R1) guideline.

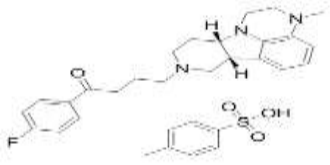
Keywords: ICH Q2 (R1) guideline, Lumateperone tosylate, Quality by Design, RP-HPLC, validation.

INTRODUCTION:

Lumateperone tosylate is a recently approved 2nd-generation antipsychotic recently used for the therapy of schizophrenia. It has a special receptor attachment form and vary from other antipsychotics in that it modulates glutamate, serotonin, and dopamine, which are all neurotransmitters that put up to the pathophysiology of schizophrenia. Lumateperone, also called as ITI-007, is an atypical antipsychotic that has showen to be effectual in the therapy of schizophrenia. Lumateperone's receptor attachment form is special, permiting it to resovlve

schizophrenia-related symptoms while diminishing undesirable effects. In difference to other second-generation antipsychotics such as lurasidone and bexpiprazole, lumateperone act as a incomplete agonist and as an antagonist at prior- and aftersynaptic dopamine (D2) receptors sensory organs , respectively. Patients with medium ordrastic liver insufficiency (Child-Pugh class B or C) tend to have more plasma concentrations of lumateperone than those with mild hepatic function. For this reason, patients with moderate or severe liver insufficiency should be given fifty percent the suggested daily dosage. There is more to learn about the pathophysiology of schizophrenia; however, dopamine deformity, specifically in the prefrontal and mesolimbic brain parts, are congrous in people with schizophrenia. In addition to dopamine, other neurotransmitters such as serotonin, glutamate, GABA, and acetylcholine are thought to play a role. Lumateperone is singular among second-generation antipsychotics based on its target profile and dopamine D2 receptor occupancy. Unlike other antipsychotics, lumateperone has partial agonist activity at presynaptic dopamine (D2) receptors, resulting in reduced presynaptic release of dopamine, and antagonistic activity at postsynaptic dopamine (D2) receptors. These featured permit lumateperone to efficiently decrease dopamine waving. Lumateperone also targets dopamine (D1) receptors, and a helpful secondary result of D1 stimulation is increased glutamatergic N-methyl-D-aspartate (NMDA) GluN2B receptor phosphorylation. This is important since NMDA-mediated glutamate wavings appears to be harmed in patients with schizophrenia. At last, lumateperone is capable of modulating serotonin by preventing serotonin transporters (SERT) and by behaving as a 5-HT2A receptor antagonist.

General drug profile: Lumateperone tosylate



Category	atypical antipsychotic
Chemical Name	1-(4-fluorophenyl)-4-[(10R,15S)-4-methyl-1,4,12-triazatetracyclo[7.6.1.0^{5,16}.0^{10,15}]hexadeca-5,7,9(16)-trien-12-yl]butan-1-one; 4-methylbenzene-1-sulfonic acid
Molecular Formula	$C_{31}H_{36}FN_3O_4S$
Molecular Weight	565.7 g/mole
Odour	Odourless

Description such	White to off white powder. Solubility soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), it is sparingly soluble in aqueous buffers
Pka	8.47 (Strongest Basic)
Melting point	182-183°C
Protein binding	Lumateperone is approximately 97.4% plasma protein bound
Uses	Lumateperone is a recently FDA-accept, first in class drug used for the treatment of schizophrenia. It is abtainable in 42mg capsule for a once a daily.

Mechanism of Action:
Lumateperone work as a sense organ antagonist of the 5-HT2A receptor and antagonizes number of dopamine receptors (D1, D2, and D4) with decereasing affinity. It has mild serotonin transporter reuptake prevention. It has including off-target antagonism at alpha-1 receptors in the absence of considerable antimuscarinic or antihistaminergic effects, limiting complication associated with other atypical antipsychotics.

Pharmacokinetics: later receipts the medication drug by mouth, lumateperone distributed hogher plasma concentrations within 1–2 hours and has a final removal half-span of 18 hours. Lumateperone is a substrate for number of metabolic enzymes, including several glucuronosyltransferase (UGT) isoforms (UGT1A1, 1A4, and 2B15), aldo-keto reductase (AKR) isoforms (AKR1C1, 1B10, and 1C4), and cytochrome P450 (CYP) enzymes (CYP3A4, 2C8, and 1A2). Lumateperone does not show numerous prevention of any common CYP450 enzymes. It is not a substrate for p-glycoprotein

MATERIALS AND METHODS:

Materials: Lumateperone tosylate was a gift sample from Swapnroop Research Pvt. Ltd. Lumateperone tosylate capsules used were 42 mg from Lupin Pharmaceuticals. HPLC-grade methanol, water, and OPA are used.

Instruments: Waters corp HPLC, column Phenomenex C18 , UV – visible detector, manual inject port, breeze software, precision balance, digital pH meter, Digital ultra sonicator.

Preparation of Mobile Phase:
Methanol: 0.1% Ortho Phosphoric Acid (45:55 v/v)

Preparation of Standard Stock Solution: 25 mg Lumateperone tosylae was exactly measured and shift into a 25 ml volumetric flask. prepare volume up to the sign with the diluent solvent to get a concentration of 1000 micro gram per ml.. Through this mixture, develop another dilution.

Test solution preparation: Take hold of 20 capsules, every capsule carry 42 mg of lumateperone tosylate. The capsules were stamp out to a little powder, and theamount of powder similar to 25 mg of lumateperone tosylate was measured and addition in a 25-ml volumetric flask dilute with methanol and vibrateto make a clear mixtre. The solution was pass through by using a membrane filter and gas descharge.

VALIDATION PARAMETERS

A) Linearity: undisclosed conc. describe that aresimilar to the concentration of test piece in samples within a given limit called as linearity. Determination: get hold of 6 unlike concentrations, and each hold of 3copies. Draw up graph conc. Vs. Area and enumerate correlation coefficient and %RSD

B) Accuracy (%Recovery): Be the possession of undisclosed conc. Solutions outline acquire by that method to the notice value are called as accuracy. The% recovery examined by puttinrg known conc. of STD solution opposed to test solution.

C) Precision: The numeral of trial solutions of the same sample giving the similr results is called as precision. Through this measured SD and RSD Method for Precision: Determination: grasp either 3 dissimilar conc. and each prepare 3opies or grasp 6 reproduce of the identical concentration and measure precision.

D) Robustness: It is the calibrate of magnitude of the method to persist unaltered by a smaller but intentional planned contrast in the method structure and gives a indication of its continual under normal usage. Determination: Calculated by exchange unalike variables that effect method presentation within a restriction. The undisclosed conc. solution and known conc. solution was inserted under variable chromatographic condition as shown below.

E) Limit of Detection: The bottommost conc. of the substance undergoing analysis in the sample that the method can find but not necessarily measure under the given experimental state simply shows that the sample is below or above certain range. Limit test prescribed as percentage or as parts per million. The limit of detection will not only conditional on the procedure of analysis but also on the variety of device.

F)Limit of Quantitation: The small conc. of test sample can be measured below the given experimental state. The S/N ratio should not be less than 10 and RSD $\leq$ 2%.

PLAN OF WORK

A) Literature view B) Procurement of drug C) Preliminary characterization of model drugs Identification of drug: organoleptic properties: color, odor, taste, and appearance only Melting point detection UV spectrophotometer analysis D) Development of RP-HPLC method by using QbD approach and its optimizations E) Validation of proposed HPLC method Method will be validated using ICH Q2 (R1) guidelines Accuracy, Precision, Linearity, and Range, Limit of Detection, Limit of Quantitation, Robustness, Result and Discussion

C) Preliminary Characterization of model drugs

Colour: White off white, odourless, Amorphous powder
Melting point: 182°C-183°C.

Lumateperone Tosylate UV spectra (20 PPM): Methanol:Water (50:50)

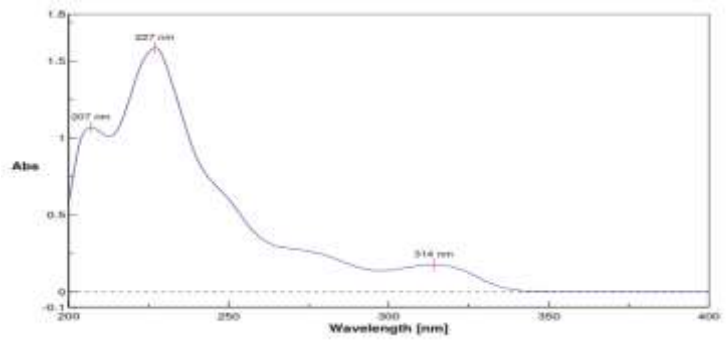
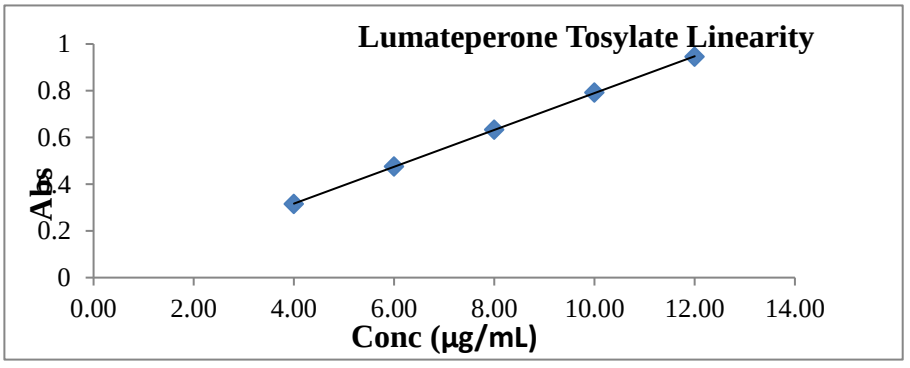


Fig 1 UV spectra of Lumateperone Tosylate

Observation: The standard solution was examine from 400 nm to 200 nm. Wavelength of maximum absorption was calculated for drug. Lumateperone Tosylate visible absorbance at 314, 227 and 207 nm. 227 nm look at as an analytical wavelength for another resolution.

Calibration curve:



Graph 1 Calibration curve of Lumateperone Tosylate

Correlation coefficient (R^2): 0.9999 Slope: 0.07886

METHOD DEVELOPMENT BY RP – HPLC

Trial 1 Chromatographic Conditions

Mode	:	Isocratic mode
Standard solution	:	Lumateperone Tosylate 100 PPM
Detector	:	U.V. Detector
Wavelength	:	227 nm
Column Make	:	Phenomenex
Column Chemistry	:	C18
Column Dimension	:	250 mm X 4.6 mm i.d., 5µm
Column Oven temperature	:	35º C
Injection Volume	:	20µl
Mobile phase	:	**Methanol: Water (70:30 % v/v)**
Flow Rate	:	1.0 ml/min

Chromatogram:

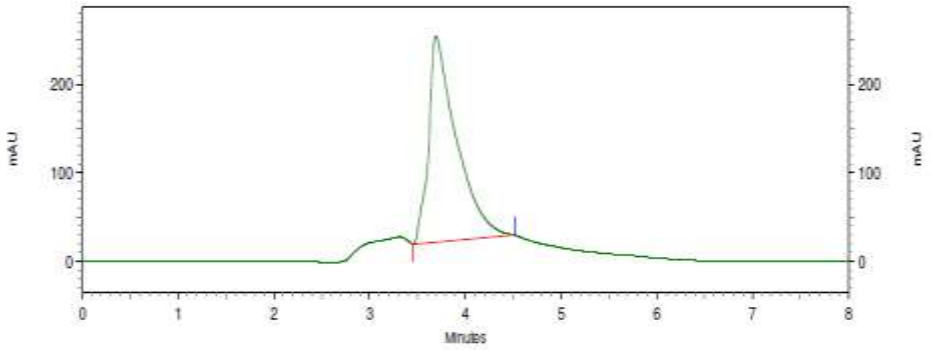


Fig 2 Typical chromatogram of Trial 1

Observation: Lumateperone Tosylate eluted at 3.69 minutes with unacceptable chromatography. Peak shape is not sharp (Theoretical plates = 864)

Conclusion: Method rejected.

Trial 2: Chromatographic Conditions:

Mode	:	Isocratic mode
Standard solution	:	Lumateperone Tosylate 100 PPM
Detector	:	U.V. Detector
Wavelength	:	227 nm
Column Make	:	Phenomenex
Column Chemistry	:	C18

Column Dimension	:	250 mm X 4.6 mm i.d., 5µm
Column Oven temperature	:	35º C
Injection Volume	:	20µl
Mobile phase	:	**Acetonitrile: Water (70:30 % v/v)**
Flow Rate	:	1.0 ml/min

Chromatogram:

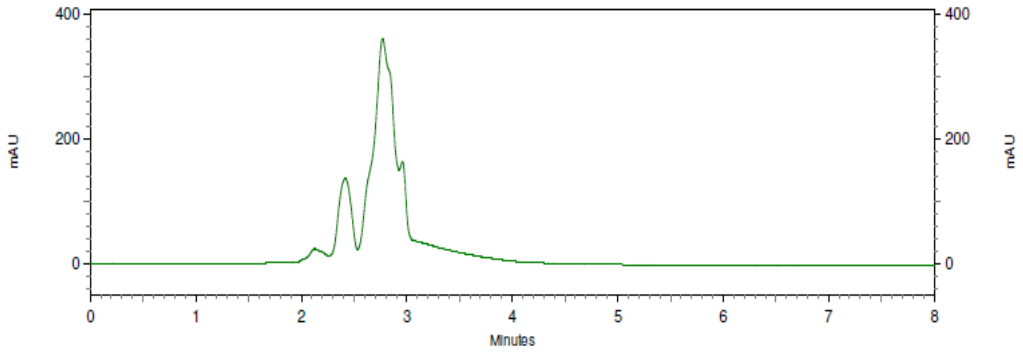


Fig 3Typical chromatogram of Trial 2

Observation: Lumateperone Tosylate eluted with unacceptable chromatography. Unpredictable chromatography observed.(R.T. 2.7 min)

Conclusion: Method rejected.

Trial 3: Chromatographic Conditions:

Mode	:	Isocratic mode
Standard solution	:	Lumateperone Tosylate 100 PPM
Detector	:	U.V. Detector
Wavelength	:	227 nm
Column Make	:	Phenomenex
Column Chemistry	:	C18
Column Dimension	:	250 mm X 4.6 mm i.d., 5µm
Column Oven temperature	:	35º C
Injection Volume	:	20µl
Mobile phase	:	**Methanol: 0.1% OPA in water (70:30 % v/v)**
Flow Rate	:	1.0 ml/min

Chromatogram:

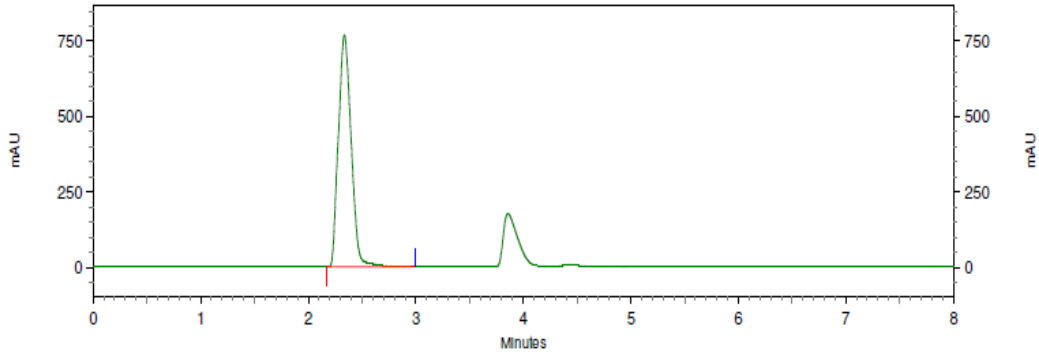


Fig 4 Typical chromatogram of Trial 3

Observation: Lumateperone Tosylate eluted at 2.34 minutes with acceptable chromatography. Peak is eluted too early which is not suitable for force degradation study

Conclusion: Method rejected.

Trial 4: Chromatographic Conditions:

Mode	:	Isocratic mode
Standard solution	:	Lumateperone Tosylate 100 PPM
Detector	:	U.V. Detector
Wavelength	:	227 nm
Column Make	:	Phenomenex
Column Chemistry	:	C18
Column Dimension	:	250 mm X 4.6 mm i.d., 5µm
Column Oven temperature	:	35º C
Injection Volume	:	20µl
Mobile phase	:	**Methanol: 0.1% OPA in water (50:50 % v/v)**
Flow Rate	:	1.0 ml/min

Chromatogram:

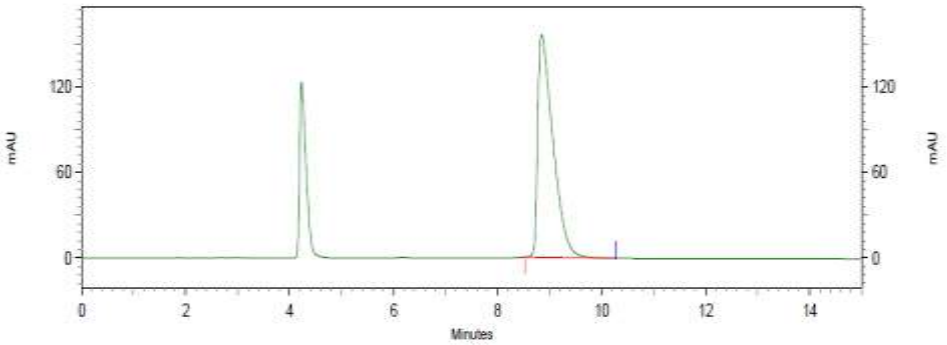


Fig 5 Typical chromatogram of Trial no. 4

Observation: Lumateperone Tosylate eluted at 8.85 minutes with acceptable chromatography. R.T. of Lumateperone is suitable for force degradation study.

Conclusion: Method can be accepted and use to apply DOE.

In chromatogram of 4th trial, we got two peaks. One of which may be of tosylate. In order to confirm these peaks, sample analyzed on mass analyzer to determine the name of peak.

Results:

1) **Mass spectra of first peak eluted at 4.15 minutes:**

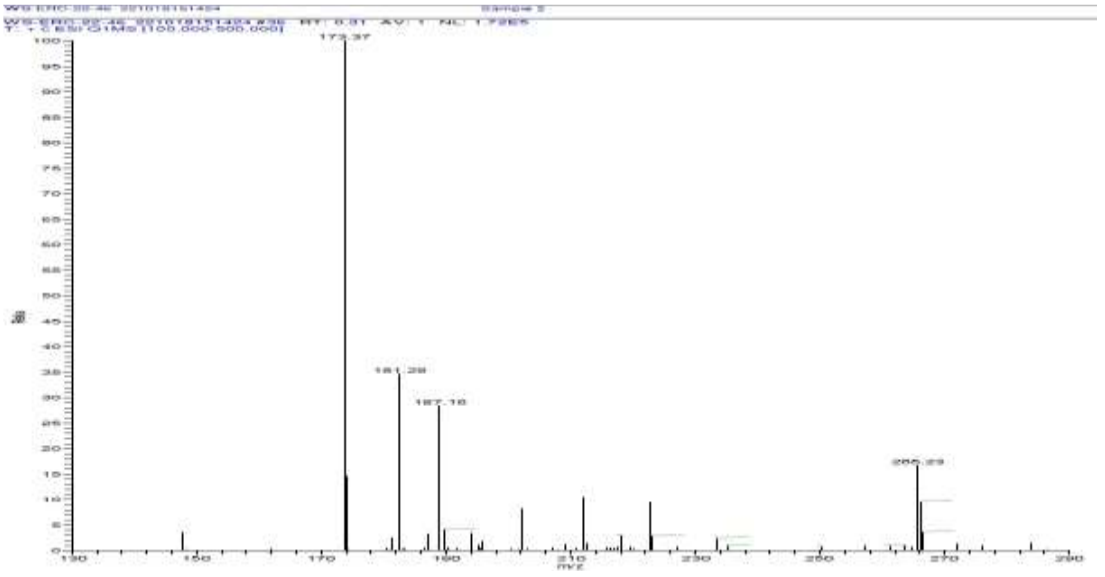


Fig 6 Mass spectra of first peak

1) Mass spectra of Second peak eluted at 8.85 minutes:

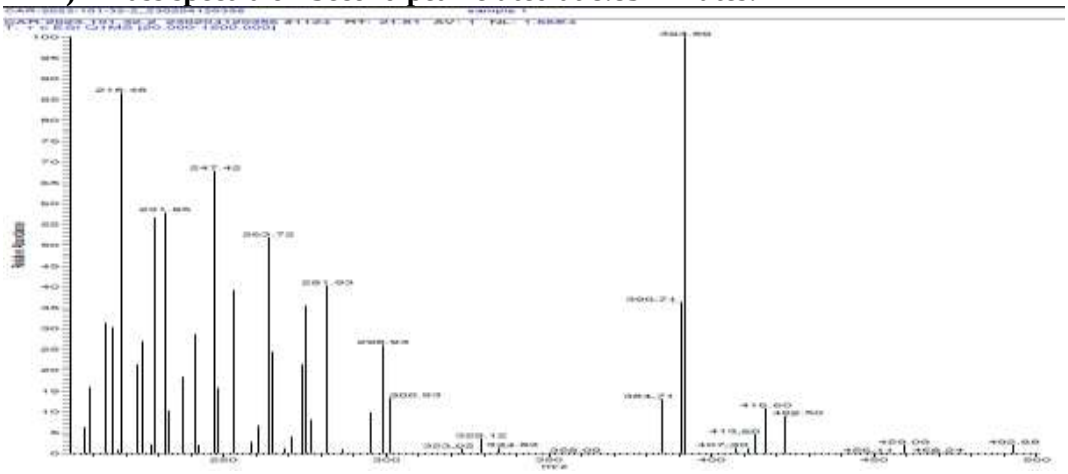


Fig 7 Mass spectra of second peak

Observation:

Mass spectra of first peak: m/z is 173.37. Tosylate molecular weight is 172.20 g/mol. **Mass spectra of Second peak**: m/z is 394.89. Lumateperone molecular weight is 393.51 g/mol.Mass spectra observed on basis of principle of M+1 rule on mass.

Conclusion: First peaks is of tosylate and second peak is of Lumateperone.

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

Entire action of mathematical calculation for the present optimization study and statistical analysis were carrying out utilization of Design Expert® software (Design Expert version 7.0.0; State-Ease Inc., Minneapolis, MN, USA).

Preparation of standard solutions to inject in DOE runs:

100 ppm solution was used for DOE run.

Table 1 Translation of coded levels in actual values

Level of Variable	**Range of Factors**		
	Methanol (%v	**Flow Rate (mL/min)**	**Column oven temperature (°C)**
Low Level (-1)	45	0.8	30
Medium Level (0)	50	1.0	35
High Level (1)	55	1.2	40

Table 2 Variables differentiation

Variable level in actual form			
Run	X1	X2	X3
1	50	1	35
2	45	1.2	35
3	55	0.8	35
4	45	1	30
5	50	1	35
6	50	0.8	40
7	45	1	40
8	50	1.2	30
9	50	1.2	40
10	55	1	40
11	45	0.8	35
12	55	1.2	35
13	55	1	30
14	50	1	35
15	50	0.8	30

Table 3 Result summary of DOE trials

Runs	**Factor1**	**Factor 2**	**Factor3**	**Response 1**	**Response 2**	**Response 3**
	A: % Methanol	**B: Flow rate**	**C: COT (°C)**	**Retention time (RT)**	**Asymmetry**	**TP**
1	50	1	35	7.03	1.65	5894
2	45	1.2	35	8.75	1.7	6715

3	55	0.8	35	5.31	1.41	8545
4	45	1	30	12.04	1.82	6830
5	50	1	35	7.03	1.65	5886
6	50	0.8	40	7.88	1.77	7671
7	**45**	**1**	**40**	**9.62**	**1.72**	**7148**
8	50	1.2	30	6.07	1.6	6110
9	50	1.2	40	5.34	1.66	6362
10	55	1	40	4.03	1.35	7391
11	45	0.8	35	13.6	1.87	7459
12	55	1.2	35	3.54	1.32	6465
13	55	1	30	4.50	1.40	7393
14	50	1	35	7.03	1.64	5705
15	50	0.8	30	8.98	1.79	7551

Selection of Optimized method:

Trial no. 7 selected as optimized chromatography, as it has Optimum R.T., Good asymmetry and theoretical plates.

It has R.T. at about 10 minutes. It's suitable for Force degradation study as there may be possibility of eluting impurities too early if we choose another trial which shows R.T. at about 5-7 minutes. Trial no. 2 is also suitable for further study but back pressure is high as compare to trial no 7, as trial 2 has flow rate. of 1.2 mL/min.

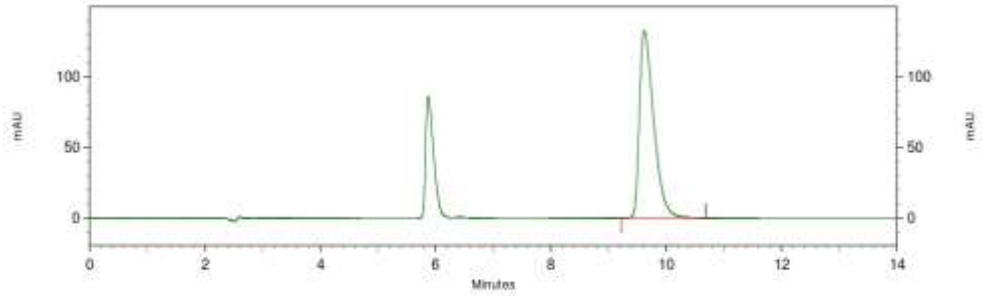


Fig 8 Optimized Chrogram of Lumateperone Tosylate

A) Results for the Retention time of DOE:

1. **Fit Summary:** After entering the data in Design-Expert software, fit summary applied to the data after which the "quadratic vs 2FI" was suggested by the software.

Table 4 Fit summary table for R.T. of DOE

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Mean vs Total	817.70	1	817.70			
Linear vs Mean	109.64	3	36.55	76.42	< 0.0001	
2FI vs Linear	3.36	3	1.12	4.70	0.0356	
Quadratic vs 2FI	1.58	3	0.53	7.99	0.0236	Suggested
Cubic vs Quadratic	0.33	3	0.11	63660000.00	< 0.0001	Aliased
Residual	0.00	2	0.00			
Total	932.61	15	62.17			

2. **ANOVA for retention time of DOE:**

The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. The results of ANOVA for the retention time of DOE are as following Table.

Table 5 ANOVA table for a retention time of DOE

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	114.41	6	19.07	309.53	< 0.0001	significant
A-METHANOL	88.64	1	88.64	1438.97	< 0.0001	
B-FLOW RATE	18.21	1	18.21	295.61	< 0.0001	
C-COT	2.78	1	2.78	45.21	0.0001	
AB	2.37	1	2.37	38.50	0.0003	
AC	0.95	1	0.950625	15.43	0.0044	
A^2	1.45	1	1.445860	23.47	0.0013	
Residual	0.49	8	0.061603			
Lack of Fit	0.49	6	0.082137			
Pure Error	0.00	2	0.000000			

Cor Total	114.90	14				

The Model F-value of 309.53 implies the model is significant. There is only a 0.01% 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, C, AB, AC, A^2 are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant

3. Fit Statistics for R.T. of DOE

Table 6 Fit Statistics for R.T. of DOE

Std. Dev.	0.248	R-Squared	0.9957
Mean	7.383	Adj R-Squared	0.9925
C.V. %	3.362	Pred R-Squared	0.9769
PRESS	2.656	Adeq Precision	57.06

The "Pred R-Squared" of 0.9769 is in reasonable agreement with the "Adj R-Squared" of 0.9925. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable.Ratio of 57.06 indicates an adequate signal. This model can be used to navigate the design space.

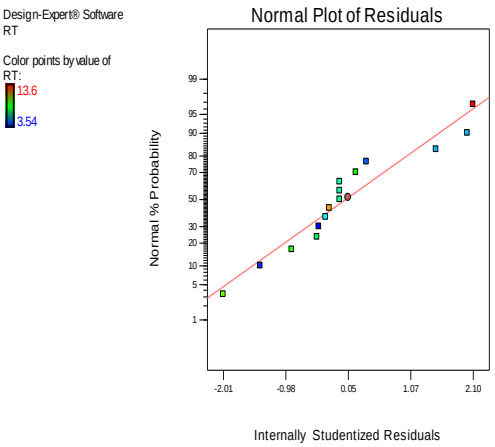
4. Final Equation in Terms of coded Factors for R.T. of DOE:

Table 7 Final Equation

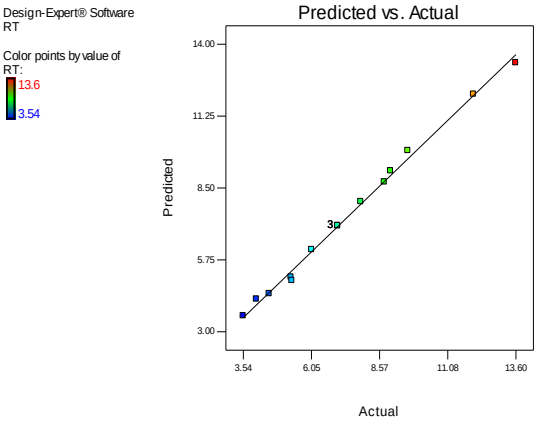
RT	=
7.0514	
-3.3288	* A
-1.5088	* B
-0.5900	* C
0.7700	* A * B
0.4875	* A * C
0.6223	* A^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor.

5. Graphical Presentation: Diagnostics of R.T. for DOE

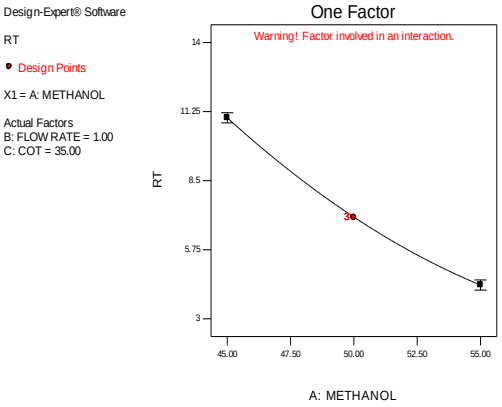


Graph 2:Normal % Probability for DOE of R.T.

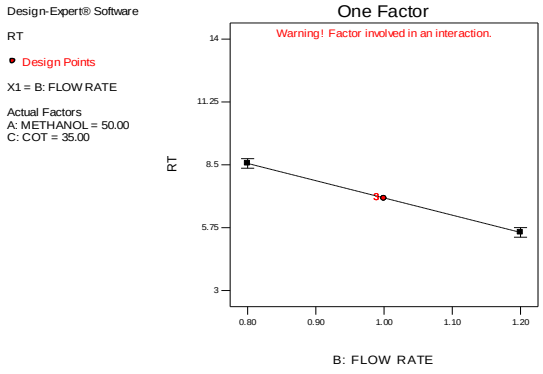


Graph 3: Predicted Vs Actual for DOE of R.T.

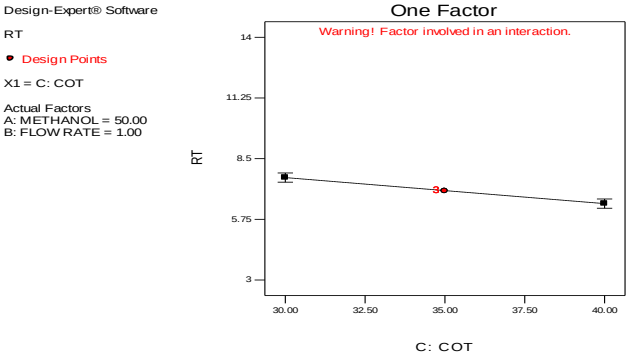
6. Model Graphs of Retention time: One-factor Graphs of Retention time for DOE:



Graph 4: Effect of % methanol in mobile phase on R.T.



Graph 5: Effect of Flow rate of mobile phase on R.T.



Graph 6: Effect of C.O.T on R.T.

Conclusion: Percentage of methanol in mobile phase has high impact on R.T. of Lumateperone. As % methanol in mobile phase increases, R.T. get decreases.

As Flow rate increases, R.T. get decreases. As COT increases, R.T. get decreases.

B) Results for the asymmetry of DOE:

1. **Fit Summary:** After entering the data in Design-Expert software, fit summary applied to the data after which the "Quadratic vs 2FI" was suggested by the software.

Table 8 Fit summary table for asymmetry of DOE

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Mean vs Total	39.53	1	39.53			
Linear vs Mean	0.37	3	0.12	24.02	< 0.0001	
2FI vs Linear	0.00	3	0.00	0.19	0.8988	
Quadratic vs 2FI	0.05	3	0.02	15.71	0.0056	Suggested
Cubic vs Quadratic	0.01	3	0.00	50.25	0.0196	Aliased
Residual	0.00	2	0.00			
Total	39.96	15	2.66			

2. ANOVA for Asymmetry of DOE:

The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. The results of ANOVA for the asymmetric factor of DOE are as following Table

Table 9 ANOVA table for asymmetry of DOE

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	0.41	3	0.14	93.15	< 0.0001	significant
A-METHANOL	0.33	1	0.33	224.47	< 0.0001	
B-FLOW RATE	0.04	1	0.04	26.49	0.0003	
A^2	0.04	1	0.04	28.49	0.0002	
Residual	0.02	11	0.00			
Lack of Fit	0.02	9	0.001801	54.03	0.0183	
Pure Error	0.00	2	0.000033			
Cor Total	0.43	14				

The Model F-value of 93.15 implies the model is significant. There is only a 0.01% 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, A^2 are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant.

3. Fit Statistics for Asymmetry for DOE

Std. Dev.	0.038	R-Squared	0.9621
Mean	1.623	Adj R-Squared	0.9518
C.V. %	2.369	Pred R-Squared	0.9316
PRESS	0.029	Adeq Precision	27.56

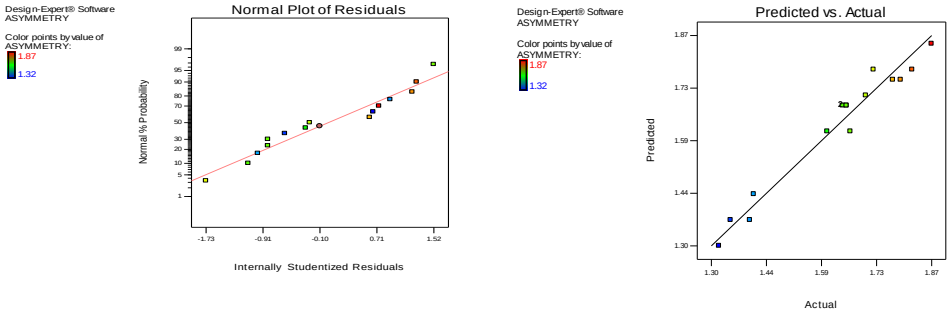
The "Pred R-Squared" of 0.9316 is in reasonable agreement with the "Adj R-Squared" of 0.9518."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 27.56 indicates an adequate signal. This model can be used to navigate the design space.

4. Final Equation in Terms of Coded Factors of Asymmetry for DOE:

Table 10: Final equation

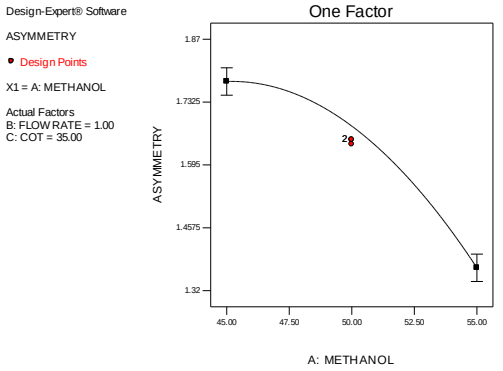
ASYMMETRY	=
1.6800	
-0.2038	* A
-0.0700	* B
-0.1063	* A^2

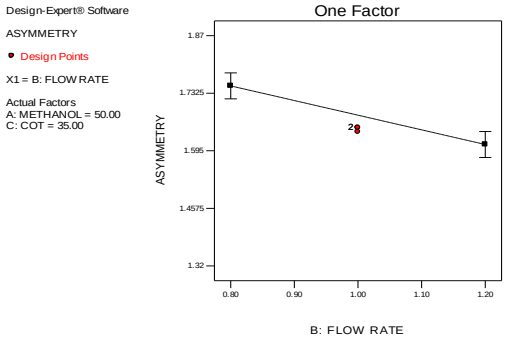
5. Graphical Presentation: Diagnostics of Asymmetry for DOE



Graph 6: Normal % Probability for DOE of Asymmetry Graph 7:Predicted Vs Actual for DOE of Asymmetry

6. Model Graphs of Asymmetry: One-factor Graphs of Asymmetry for DOE





Graph 8: Effect on % Methanol in Mobile phase on Asymmetry Graph 9:Effect on Flow rate on Asymmetry

Conclusion: Percent of Methanol in mobile phase has slight curvature impact on Asymmetry. As Methanol in mobile phase increases, Asymmetry get decreases.

Flow rate is also having impact on asymmetry, as F.R. increases asymmetry decreases .

C)Results for Theoretical plates DOE:

1. **Fit Summary**: After entering the data in Design-Expert software, fit summary applied to the data after which the "Quadratic vs 2FI" was suggested by the software.

Table 11 Fit Summary for theoretical plates of DOE

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Mean vs Total	708984375.00	1	708984375.00			
Linear vs Mean	4279873.00	3	1426624.33	3.20	0.0661	
2FI vs Linear	476180.00	3	158726.67	0.29	0.8338	
Quadratic vs 2FI	4403067.33	3	1467689.11	305.29	< 0.0001	Suggested
Cubic vs Quadratic	1189.00	3	396.33	0.03	0.9890	Aliased
Residual	22848.67	2	11424.33			
Total	718167533.00	15	47877835.53			

2. ANOVA for Theoretical plates of DOE:

Linear Model selected for analysis.

The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. The results of ANOVA for the **theoretical plates** of DOE are as follows,

Table 12 ANOVA table for Theoretical plates of DOE.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	9129164.33	7	1304166.33	169.08	< 0.0001	significant
A-METHANOL	337020.50	1	337020.50	43.69	0.0003	
B-FLOW RATE	3883684.50	1	3883684.50	503.50	< 0.0001	
C-COT	59168.00	1	59168.00	7.67	0.0277	
AB	446224.00	1	446224.00	57.85	0.0001	
A^2	2777601.64	1	2777601.64	360.10	< 0.0001	
B^2	1330708.10	1	1330708.10	172.52	< 0.0001	
C^2	904098.56	1	904098.56	117.21	< 0.0001	
Residual	53993.67	7	7713.38			
Lack of Fit	31145.00	5	6229.00	0.545239693	0.7473	

Pure Error	22848.67	2	11424.33			
Cor Total	9183158	14				

The Model F-value of 169.08 implies the model is significant. There is only a 0.01% 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, C, AB, A^2, B^2, C^2 are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant.

3. Fit Statistics for theoretical plates **of DOE**

Table 13: Fit Statistics for theoretical plates of DOE

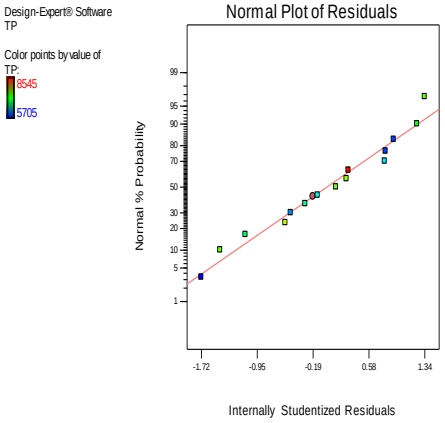
Std. Dev.	87.826	R-Squared	0.9941
Mean	6875.000	Adj R-Squared	0.9882
C.V. %	1.277	Pred R-Squared	0.9803
PRESS	180771.500	Adeq Precision	42.15

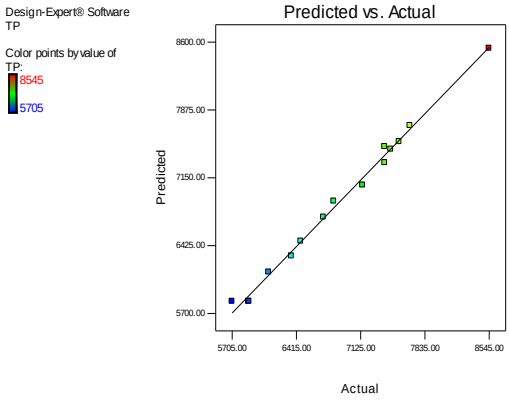
The "Pred R-Squared" of 0.9803 is in reasonable agreement with the "Adj R-Squared" of 0.9882.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable.

Ratio of 42.15 indicates an adequate signal. This model can be used to navigate the design space.

5. Graphical Presentation: Diagnostics of theoretical plates for DOE

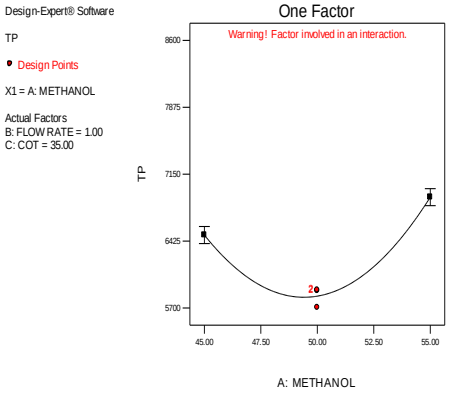


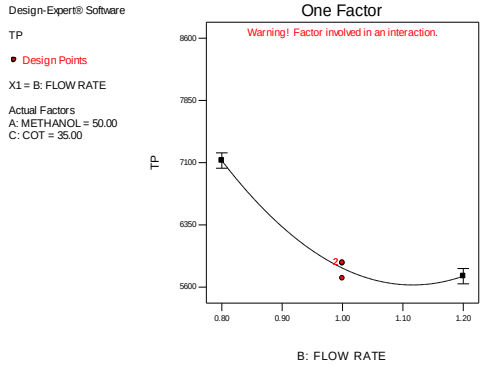


Graph 10 Normal % Probability for DOE of **TP**

Graph 11 Predicted Vs Actual for DOE of **TP**

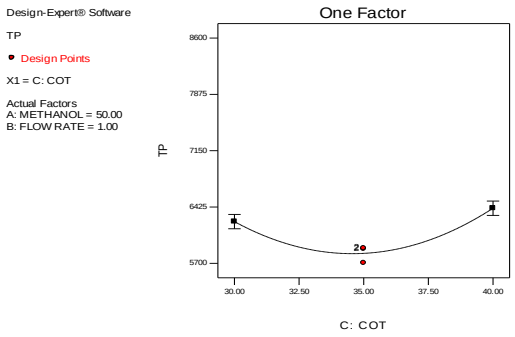
6. Model Graphs of Theoretical plates: One-factor Graphs of Theoretical plates for DOE:





Graph 12 Effect of % Methanol in mobile phase on TP

Graph 13 Effect of Flow rate on TP



Graph 14 Effect of COT on Theoretical plates

Conclusion: Percent of Methanol in mobile phase has curvature effect on Theoretical plates. As Percent of Methanol increases Theoretical plates increases.

As Flow rate is also having slights curvature effect on Theoretical plates as Flow rate increases, Theoretical plates decreases. As COT is also having slights curvature effect on Theoretical plates as COT increases, Theoretical plates increases.

We have selected DOE trial no.7 as optimized chromatography which has following parameters and actual results

RESULTS AND DISCUSSION:

Table 15 Optimized Chromatography

Runs	**Factor1**	**Factor 2**	**Factor3**	**Response 1**	**Response 2**	**Response 3**
	A: % Methanol	**B: Flow rate**	**C: COT (°C)**	**Retention time (RT)**	**Asymmetry**	**TP**
7	45	1.00	40	9.62	1.72	7148

By entering trial no. 7 results in optimization and checked for solutions as follows:

Name	**Goal**	**Target value**
A: Methanol	Target->	45
B: FR	Target->	1.00

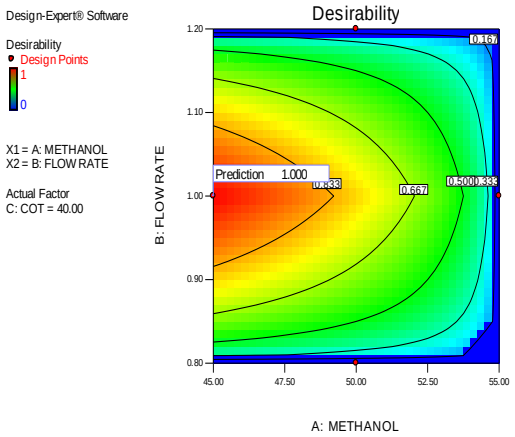
C: C.O.T.	Target->	40
R.T.	Range	-
Asymmetry	Range	-
Theoretical plates	Range	-

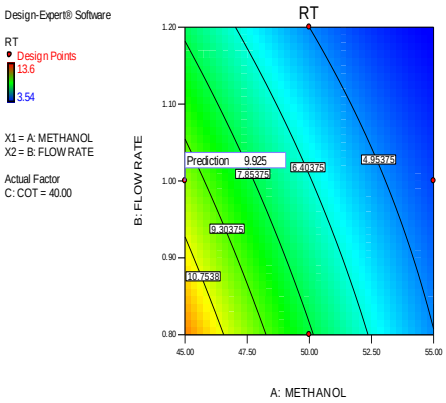
Table 17 Optimization solutions: Result of optimization for DOE

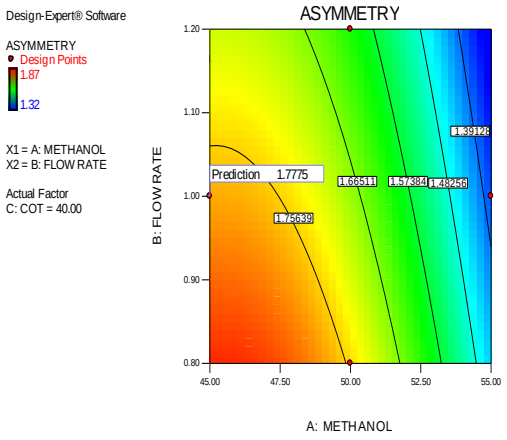
Number	Methanol	FLOW RATE	COT	RT	Asymmetry	TP	Desirability	
1	45.00	1.00	40	9.925	1.78	7071	1.0000	Selected
2	45.04	1.00	40	9.896	1.78	7060	0.9988	
3	45.00	1.00	40	9.952	1.78	7076	0.9960	
4	45.14	1.00	40	9.809	1.78	7028	0.9952	
5	45.00	1.00	39.58	10.015	1.78	6985	0.9859	

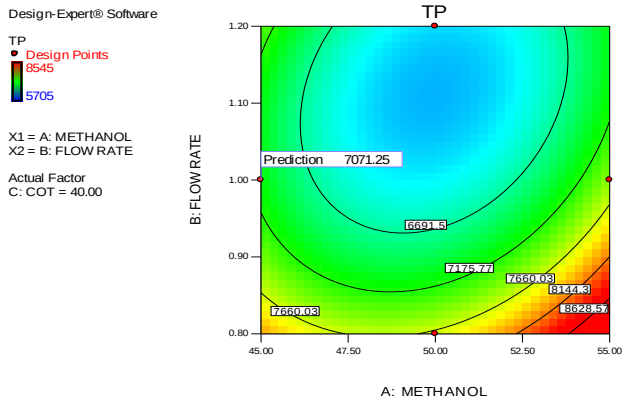
Conclusion: Used DOE model predict almost same chromatography results as that of trial no. 7 with the desirability 1.00 . Solution no.1 shows almost same parameters with the actual results of trial no. 7 (±10%). Hence proposed Box behnken surface methodology model found fit for developed chromatographic method and it can be used to predict dependent variable within a design space.

Design space:









Graph 16 Design space for Desirability, R.T, Asymmetry and Theoretical plates**.**

Table 18 Optimized chromatography method is as follows

Parameter	**Description**
Mode	Isocratic
Column Name	Phenomenex C18**,** 250 mm X 4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	227 nm
Column Oven temp	40ºC
Mobile Phase	Methanol : 0.1% OPA in water (45:55%V/V)
Flow Rate	1.00 ml/min

Validation:

System Suitability test: (100PPM Std. solution)

Table 19 Observation Summary of System suitablity

Sr No.	**Standard solution**	**Area**	**Asymmetry**	**Theoretical plates**
1	Standard-1	40635284	1.72	7186
2	Standard-2	40638951	1.72	7149
3	Standard-3	40629765	1.72	7162
4	Standard-4	40627583	1.71	7203
5	Standard-5	40610586	1.72	7139
Mean		**40628434**	**1.72**	**7168**
STD Dev		**40628434**		
% RSD		**40628434**		

Acceptance Criteria:

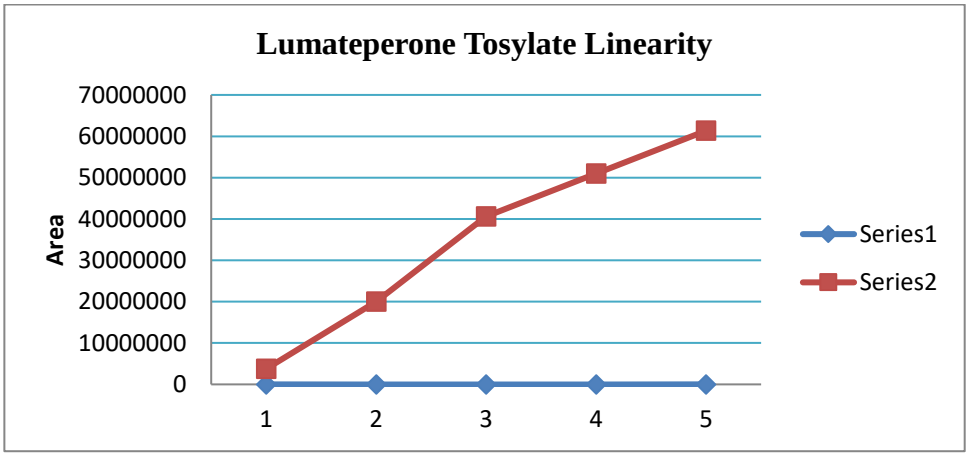
1. RSD should NMT 2.0 % for six duplicate injections of known conc. solution
2. USP Tailing Factor NMT 2.0.
3. The Plate Count more than 2000.

Conclusion: System suitability pass the test.

A) Linearity:

Table 20 Dilution table for linearity of Lumateperone Tosylate

Level	**Conc (µg/mL)**	**Area**	**Mean**	**STD DEV**	**% RSD**
10%	10	3767049	3767153	1318.599	0.035
		3768521			
		3765890			
50%	50	19997337	19999008	43337.178	0.217
		19956531			
		20043157			
100%	100	40627767	40630455	3878.099	0.010
		40628698			
		40634901			
125%	125	50978522	50958864	18602.295	0.037
		50956534			
		50941537			
150%	150	61343762	61348885	5273.443	0.009



Graph 17 Linearity curve Lumateperone tosylate

Results: Correlation coefficient: 0.9999 Intercept: -455968.8459 **Slope:** 411457.9523

Acceptance criteria: correlation coefficient ≥ 0.98

Conclusion: Regression coefficient was found well within acceptance limit for proposed range.

Accuracy (%Recovery):

Table 21 Observation summary of Accuracy

Level (50 %)	**Area**	**Recovered conc**	**Added conc**	**% Recovery**	**Mean Recovery**	**% RSD**	**Overall Recovery**	**% RSD for over all recvoery**
50	20522469	50.513	51.000	99.05	**99.81**	**0.846**	**99.43**	**0.627**
	20665497	50.865	50.500	100.72				
	20450064	50.334	50.500	99.67				
100	40625604	99.993	100.000	99.99	**99.49**	**0.513**		
	40609977	99.955	101.000	98.97				
	40635626	100.018	100.500	99.52				
150	60553284	149.042	150.500	99.03	**98.97**	**0.213**		

	60578536	149.104	151.000	98.74				
	60625132	149.218	150.500	99.15				

Acceptance criteria: % Recovery- 98.0 % to 102.0 %

Conclusion: % Recovery was found well within acceptance range at all three levels.

D)Precision:

Table 22 Observation summary of Precision

Sample	**Area**	**% Assay**
Sample 1	40716482	98.25
Sample 2	40751846	99.80
Sample 3	40652164	98.58
Sample 4	40702569	98.70
Sample 5	40785149	99.89
Sample 6	40726517	99.25
Mean		**99.08**
STD DEV		**0.676267**
% RSD		**0.683**

Acceptance criteria: % Assay (Individual & mean value): 98.0 to 102.0%

% RSD for 6 samples: NMT 2.0 % Precision pass the criteria, no variation found by preparing six different samples. Results are good reproducible.

Intermediate precision:

Table 23 Observation Summary and Results

Sample	**Area**	**% Assay**
Sample 1	40759846	99.82
Sample 2	40796584	98.93

Sample 3	40814527	100.46
Sample 4	40792016	99.41
Sample 5	40658744	99.58
Sample 6	40559745	98.84
Mean		**99.51**
STD DEV		**0.5998**
% RSD		**0.603**
Precision plus Intermediate precision	**Mean**	**99.293**
	STD DEV	**0.6492**
	% RSD	**0.654**

Acceptance Criteria:
% Assay (Individual & mean value): 98 to 102%
% RSD for 6 samples: NMT 2%
% RSD for 12 samples: (Precision and Intermediate precision): NMT 2%

D) Robustness:

Determination:
Quantitated by changing different variable which affect on method performance in within limit. The unknown conc. solution and known conc. solution was injected under variable chromatographic state as shown below.

- Changes in flow rate. (±10%)
- Change in wavelength. (±3nm)
- Change in column oven temperature (± 2°C)

Table 23 Change in Wavelength ±3nm

Sr.No.	System Suitability parameter	Observations			Limits
		As such (227nm)	360nm	2524nm	
1	Peak area response	3767153	40927574	37230216	
2	Theoretical plates	7148	7241	7265	NLT 2000
3	Asymmetry	1.72	1.73	1.71	NMT 2

4	Retention time (min)	9.62	10.07	10.01	

Table 24 Changes in flow rate. (±10%)

Sr.No.	System Suitability parameter	Observations			Limits
		As such	+10%	-10%	
1	Peak area response	3767153	37067580	45505718	
2	Theoretical plates	7148	7032	7586	NLT 2000
3	Asymmetry	1.72	1.68	1.76	NMT 2
4	Retention time (min)	9.62	9.05	10.98	

Table 25 Change in column oven temperature +2°C

Sr. No.	System Suitability parameter	Observations			Limits
		As such	**+2°C**	**-2°C**	
1	Peak area response	3767153	40758465	40248579	
2	Theoretical plates	7148	7364	40248579	NLT 2000
3	Asymmetry	1.72	1.69	1.75	NMT 2
4	Retention time (min)	9.62	10.19	10.23	

E) DETECTION:

(1) Limit of Detection & Quantitation:

Table 26 Result and statistical data of LOD & LOQ of Lumateperone tosylate

Lumateperone tosylate					
Sr.no	Concentration(µg/ml)	RT (min)	Area	Plate Count	Tailing
1	10	10.96	3767153	7216	1.34
2	50	10.65	19999008	7214	1.59
3	100	10.21	40630455	7219	1.71

4	125	10.39	50958864	6822	1.76
5	150	10.24	61348885	6249	1.78
Correlation Coefficient			0.99999		
Slope			411457.95		
SD			96096.96		
LOD			0.771ppm		
LOQ			2.336ppm		

CONCLUSION:

Conclusion: In this research work, as per my intention RP-HPLC method was progress by apply QbD technique with mobile phase methanol: 0.1% OPA (45:55v/v). The flow rate was used at 1.00 ml/min, and UV observation was taken out at 227 nm. The retention time for Lumateperone tosylate was establish to be 9.62 min.

A organized approach was employ to produce an effective and sturdy method, which comprise starting with the calculation of target profile attributes, risk evaluation, design of the study plan, and validation. The work was ready by using 3^3 Box Behnken response surface designs. In this work, the interactivity of 3 factors—wavelength, column oven temperature, and flow rate composition—varies at 3 levels. The influence of such a critical process parameter on the critical quality attribute of the path analysis was deliberated. Responses in expressions of retention times and resolution were examined all over the runs in design. The RP-HPLC method was progressed for evaluation of lumateperone tosylate and validated as per ICH Q2(R1) guidelines utilizing many variables.

Moreover, the lesser solvent expending along with the less analytical run time of 10 min leads shows to a profitable and eco-friendly chromatographic plan of action. Thus, the present methodology is speedy, strongest matches, need a uncomplicated sample preparation procedure, and illustrates a forever series of steps for Lumateperone tosylate

REFERENCES

1. Jatte, K P., Masne D D., Khachane, M A., Chakole, R D., & Charde, M R. (2021). QbD Approach in Analytical Method Development: A Review. International journal of pharmacy and pharmaceutical research, 21(2), 238-56.

2. Patil, KY., Dedania, Z R., Ronak , R., Dedania., &Patel, U. (2021). QbD approach to HPLC method development and validation of Ceftriaxone sodium. Future journal Pharmaceutical Sciences, 2-10.
3. Babar, S A., & Padwal, S L. (2021). QbD approach to analytical method development and its validation for estimation of Lenvatinib in bulk and pharmaceutical formulation. International Journal of Applied Pharmaceutics , 13(5), 183-8.
4. Phadke, R., Dr. Gosar, A., Mali, R., & Patil, D. (2019). A review on quality by design approaches to analytical method development. Indo American Journal of Pharmaceutical Research, 9(7), 3044-55.
5. Bhujbal, S S., & Darkunde, S L. (2019). Analytical method development and optimization of sofosbuvir drug - A QbD approach. International Journal of Pharmaceutical Sciences and Research, 10(1), 108-16.
6. International Conference on Harmonization Hormonised Tripartite guideline, Q8(R2) Pharmaceutical Development, Part I: 2009.
7. International Conference on Harmonization Hormonised Tripartite guideline ,Q10 Pharmaceutical Quality System, 2008.
8. International Conference on Harmonization Hormonised Tripartite guideline, Q9 Quality Risk Management, 2005.
9. Jyoti J., Haque, A M D., Islam, S M A., & Islam M S. (2011). Validation and Optimization of a simple RP-HPLC method for determination of cilostazol in Human serum. Indian journal of novel drug delivery, 3(2), 143-8.
10. Patil A S., & Pethe A M. (2013). Quality by design: A new concept for development of quality pharaceuticals. International journal of pharmaceutical quality assurance, 4(2), 13-9.
11. Bajaj., M., & Nanda, S. (2015). Analytical quality by design (AQbD): new paradigm for analytical method development. International journal of development research , 5(2), 3589-99.
12. Suresh., & Rambabu.(2015). Isocratic Reversed phase Liquid Chromatographic method validation for the determination of Cilostazol in pure and formulation. International journal of pharmacy and pharmaceutical research, 4(3), 180-92.
13. International Conference on Harmonization Hormonised Tripartite guideline, Q2(R1) Validation of analytical procedures: Text and methodology 2005.
14. International Conference on Harmonization Hormonised Tripartite guideline, Q1A(R2) Stability Testing of new drug substances & Products 2003.
15. Muggu M., Nagavalli, S., Pushpa A., Deep, P B., & Naik, P. (2020). Method Development and Validation of Lemborexant drug in bulk and its Pharmaceutical dosage form by RP-HPLC. World Journal of Research, 9(14), 1372-80.
16. Kambale, S N. (2022). Development and validation of novel HPLC method for analytical evaluation of Lemborexant drug tablet dosage form. GSC Advanced Research and Reviews, 11(1), 132–43.
17. Suchitra, D., & Satyanarayana, B. (2021). A Stability Indicating Reverse Phase-HPLC Method Development and Validation for the Estimation of Rucaparib in Bulk and Pharmaceutical Dosage Form. American Journal of Analytical Chemistry, 12, 96-107.
18. Gorijavolu, V., Gupta, A K., & Chowdary Y A.(2018). A sensitive bio analytical method development and validation of rucaparib in Human plasma by LC-ESI-MS/MS. International Journal of Advance Research, 6(1), 836-43.
19. Kossataz, S. et.al. (2018). Direct Imaging of Drug Distribution and Target Engagement of the PARP Inhibitor Rucaparib.,The Journal of Nuclear medicine 59(8), 1316–20.

20. Qiong Wang et al.(2021). Characterization of Alpelisib in Rat Plasma by a Newly Developed UPLC-MS/MS Method: Application to a Drug-Drug Interaction Study. National Library of Medicine ,12.
21. Parmar, I., & Patel, Y. (2022). Recent method development by analytical techniques of new FDA approved drugs in 2021. International journal of current pharmaceutical research, 14(3), 17-21.
22. Sisindri D., & Darmamoorthy, G. (2022). Development and Validation of a New analytical method for the Determination of Belzuifan in bulk and Pharmaceutical dosage form. International journal of Pharmacy and Pharmaceutical Research 25(1), 384-94.
23. Lumateperone", December 2020, https://www.drugbank.ca/Lumateperone
24. lumateperone", December 2020, https://en.wikipedia.org/wiki/Lumateperone
25. Dhami, F R., & Dhudashia, K. (2021). Development of UV spectrophotometric and RP-HPLC method for estimation of Lumateperone in solid dosage form. International journal of All Research education and scientific methods, 9[5], 3446-63.
26. Rathod, G D., & Bagwan, L. (2023). Development and validation of RP-HPLC method for estimation of Lumateperone drug in pharmaceutical dosage form. International Journal of Novel Research and Development, 8(7), 780-91.
27. Juran, J M.(1991). Juran on Quality by Design: The New Steps for Planning Quality into Goods and Services, Simon and Schuster Adult Publishing group, New York.