Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

Analytical Method Development And Validation Of RP-HPLC Method For Estimation Of Lumateperone In Bulk And Pharmaceutical Dosage Form

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.Cite this paper as: Darshana Undre, Vaibhav Shilimkar, (2025) Analytical Method Development And Validation Of RP-HPLC Method For Estimation Of Lumateperone In Bulk And Pharmaceutical Dosage Form. *Journal of Neonatal Surgery*, 14 (32s), 3967-3978.

ABSTRACT

Objective:
To develop and validate precise, accurate, and robust analytical methods—UV-Visible Spectrophotometry and Reversed Phase High-Performance Liquid Chromatography (RP-HPLC)—for the estimation of Lumateperone Tosylate in bulk and pharmaceutical dosage forms.

Methods: The UV spectrophotometric method involved selecting 227 nm as the analytical wavelength using a 50:50 v/v water:methanol diluent. Linearity was established between 5–15 µg/mL ($R^2 = 0.9997$).

The RP-HPLC method was developed using a Phenomenex C18 column with a mobile phase of phosphate buffer (pH 6.0):methanol (55:45 v/v), a flow rate of 1.2 mL/min, and detection at 227 nm. Validation parameters such as accuracy, precision, linearity, system suitability, and robustness were assessed following ICH Q2(R1) guidelines[1].

Results: The UV method demonstrated good linearity ($R^2 = 0.9997$), recovery (98.0%–102.0%), and precision (RSD < 2%). The optimized HPLC method (retention time ~4.1 min) showed acceptable asymmetry (1.21) and theoretical plates (>7500), with consistent assay results and % RSD within limits. The methods were successfully applied for quantitative estimation of Lumateperone in capsules[2].

Conclusion: Both methods were simple, reliable, and suitable for routine quality control of Lumateperone. These findings provide an analytical foundation for future pediatric formulation development of Lumateperone, pending neonatal pharmacokinetic research[3].

Keywords: *Lumateperone Tosylate, UV-Visible Spectrophotometry, RP-HPLC, Method Validation, ICH Guidelines, Pediatric Dosage*

1. INTRODUCTION

Lumateperone, a novel antipsychotic with dopamine receptor modulating properties, is primarily indicated for the treatment of schizophrenia and bipolar depression. Despite its growing clinical use, limited analytical methodologies exist for its routine quality control, especially in novel or pediatric formulations. This study aims to establish validated UV and RP-HPLC methods for Lumateperone estimation in pharmaceutical dosage forms, supporting potential neonatal applications[4].

IUPAC name: 1-(4-fluorophenyl)-4-[(10R,15S)-4-methyl-1,4,12-triazatetracyclo[7.6.1.0^{5,16}.0^{10,15}]hexadeca-5(16),6,8-trien-12-yl]butan-1-one

Molecular Formula: $C_{24}H_{28}FN_{3O}$

Molecular weight : 393.506 g/mole

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)

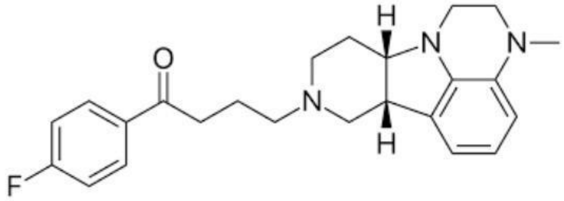

Figure 1 : molecular structure of lumateperone

MECHANISM OF ACTION

Lumateperone primarily acts by blocking the 5-HT2A serotonin receptors, contributing to its antipsychotic effects. It also shows affinity for dopamine receptors D1, D2, and D4, though at comparatively lower binding strengths. Additionally, it exhibits moderate inhibitory effects on serotonin reuptake transporters. Its pharmacological profile includes

antagonism of alpha-1 adrenergic receptors, but it does not significantly interact with muscarinic or histamine receptors, which helps minimize typical side effects seen with other atypical antipsychotics.

PHARMACOKINETICS

After oral administration, lumateperone reaches peak plasma concentrations within 1 to 2 hours. It has an elimination half-life of approximately 18 hours. The compound undergoes metabolism through several enzymatic pathways including glucuronosyltransferases (UGT1A1, UGT1A4, UGT2B15), aldo-keto reductases (AKR1C1, AKR1B10, AKR1C4), and cytochrome P450 enzymes (CYP3A4, CYP2C8, CYP1A2). Importantly, lumateperone does not significantly inhibit common CYP450 isoenzymes and is not a substrate of p-glycoprotein, reducing the risk of major drug interactions.

MATERIALS AND METHOD

Material and reagents :

Lumateperone standard drug purchased from vidhisha laboratory . The analytical study utilized high-purity reagents and calibrated instruments to ensure precision and accuracy. The reagents included water (Rankem), acetonitrile and methanol (Merck Life Science), and filtration devices such as 0.45 µm nylon membrane disc filters and PVDF syringe filters (Mdi).

Instruments and software:

For chromatographic analysis, an Agilent 1260 Infinity II HPLC system equipped with a UV detector was employed, managed through OpenLab EZ Chrome workstation software. UV spectroscopic studies were carried out using a Jasco UV-550 double-beam spectrophotometer with 10 mm matched quartz cells, operated via Spectra Manager software.

Weighing was conducted using a digital analytical balance (Aczet CY224C) with a range of 2 mg to 200 g. pH measurements were obtained using a Thermo Scientific digital pH meter (Model: Orion Star A211). Sample preparation involved the use of an ultra-sonicator (Bio-technic, 13.5-litre capacity) for uniform dispersion and solubilization.

EXPERIMENTAL WORK

1 : Solvent Selection

The solubility of Lumateperone Tosylate was evaluated at a concentration of 3 mg/mL using various solvents. Approximately 43 mg of the drug substance was weighed and subjected to sonication for 5–10 minutes in each solvent system to assess dissolution efficiency.

Water: Partial solubility was observed after sonication in 10 mL of distilled water.

Methanol: Complete solubilization occurred in 10 mL of methanol following sonication.

Water:Methanol (50:50 v/v): The drug was found to be readily soluble in a 1:1 mixture of water and methanol after similar treatment.

UV SPECTROSCOPIC METHOD.

Selection of wavelength

Preparation of standard stock solution: Lumateperone Tosylate stock solution: Weighed 36 mg Lumateperone Tosylate and dissolved in 100 mL of Diluent. (250 PPM of Lumateperone Tosylate)

Final Lumateperone Tosylate solution: Further transfer 2 mL of Lumateperone Tosylate stock solution and diluted up to 50 mL with Diluent. (10 PPM of Lumateperone Tosylate)

The API standard solutions were scanned separately between 400nm to 200nm. From the spectrum show high absorbance that select as a wavelength of drug. Selected wavelength was used for estimation of drugs. Diluent used as a Blank.

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND OPTIMIZATION

Diluent Preparation

The diluent was prepared by mixing equal volumes of distilled water and methanol (50:50 v/v) and blending thoroughly to obtain a homogeneous solvent system suitable for analytical procedures.

Preparation of Solutions

0.2 N Sodium Hydroxide: 16 g NaOH dissolved in 1000 mL water.

Buffer (pH 6.0): 2.72 g potassium dihydrogen phosphate in water, pH adjusted with NaOH and filtered.

Mobile Phase: Buffer pH 6.0 and methanol mixed in a 55:45 v/v ratio.

0.1 N HCl: Prepared by diluting 8.5 mL HCl in 1000 mL of water.

Diluent: A mixture of 0.1 N HCl and methanol in 40:60 v/v ratio.

Blank: The same diluent was used as blank**.**

Standard Stock Solution

36 mg of Lumateperone Tosylate (equal to 25 mg Lumateperone) was dissolved in 50 mL of diluent, sonicated, and diluted. A 100 ppm solution was obtained by further diluting the stock.

Sample Preparation

20 capsules were weighed, and powder equivalent to 210 mg Lumateperone was transferred to a 250 mL flask. After adding 200 mL of diluent, the sample was sonicated for 60 minutes, diluted to volume, filtered (0.45 µm PVDF), and further diluted to obtain a 50 ppm solution.

Method Optimization

Several trials were performed to select the best chromatographic conditions. The final method used a C18 column with a mobile phase of buffer and methanol, chosen based on solubility and literature. The temperature control ensured consistency in retention time and improved peak shape.

Results and Discussion

UV SPECTROSCOPIC METHOD.

Selection of wavelength:

Blank spectra:

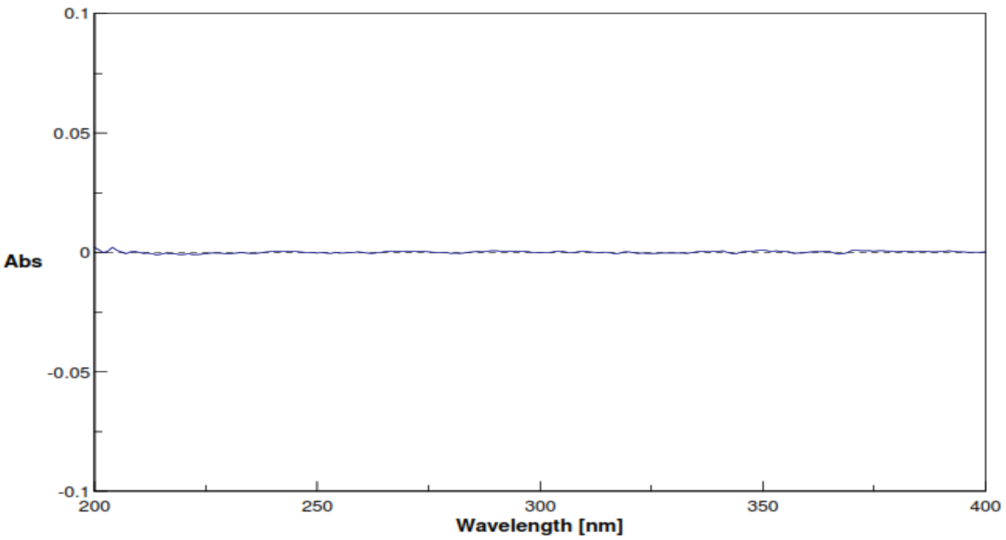

Figure 2: UV spectrum of Blank.

Standard Solution spectra:

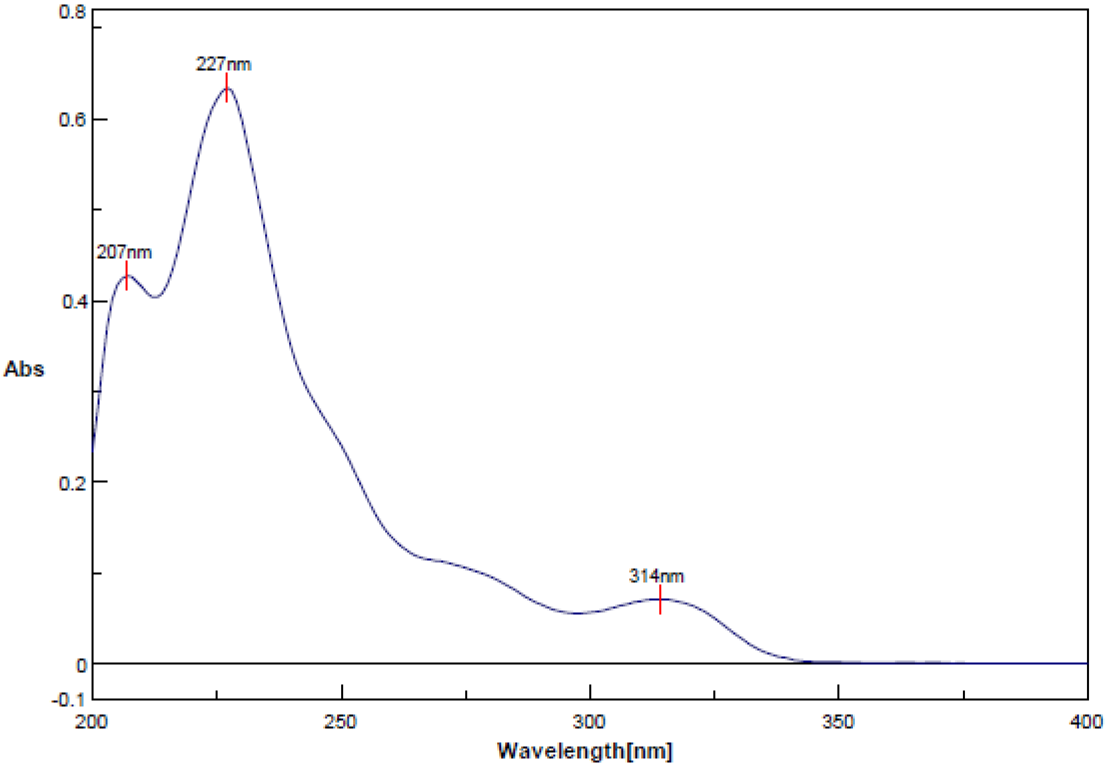

Figure 3: UV spectrum of Lumateperone Tosylate.

Observation: The standard solution was scanned from 400 nm to 200 nm. Wavelength of maximum 314, 227 and 207 nm. 227 nm considered as an analytical wavelength for further determination.

Table 1: Determination of λ max of Lumateperone Tosylate

Sr. No.	Wavelength (nm)	Absorbance
1.	227	0.6354 A^0

Conclusion:

The lambda max of Lumateperone Tosylate 227 nm is selected for further analysis.

Reverse Phase High Performance Liquid Chromatography Method Development

To optimize the RP-HPLC method for Lumateperone Tosylate, six different chromatographic conditions were tested. Trials 1 to 4 were rejected due to poor peak shapes, low theoretical plate counts, or high asymmetry. Trial 5 provided better performance, but the best result was observed in Trial 6, with a retention time of 4.1 minutes, asymmetry of 1.21, and theoretical plates of 7536—well above the acceptable threshold (NLT 2000). The optimized method used a Phenomenex C18 column, buffer pH 6.0: methanol (55:45 v/v) as the mobile phase, with a flow rate of 1.2 mL/min and detection at 227 nm. These results confirm that the final method is suitable for routine analysis with accurate and sharp chromatographic peaks.

Column	Phenomenex C18, 250 mm × 4.6 mm, 5 µm
Mobile Phase	Buffer pH 6.0 : Methanol (55:45 v/v)
Flow Rate	1.2 mL/min

Injection Volume	20 µL
Detection Wavelength	227 nm
Column Oven Temperature	40°C
Auto Sampler Temperature	25°C
Run Time	8 minutes
Seal Wash	Water : Acetonitrile (90:10 v/v)
Needle Wash	Water : Acetonitrile (10:90 v/v)

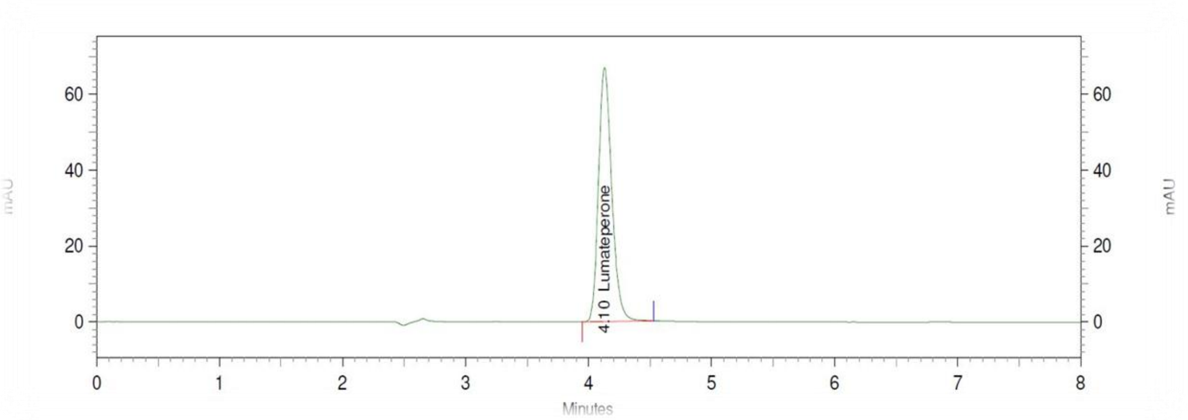

Figure 4 : typical chromatogram for lumateperone

Observation: lumateperone eluted at 4.1 minutes with acceptable chromatography (asymmetry : 1.21 and theoretical plates 7536)

Conclusion : method can be used for further analysis

METHOD VALIDATION

The following parameters were considered for the analytical method validation of title ingredients.

- System Suitability.
- Specificity.
- Linearity.
- Accuracy.
- Precision.
- Method Precision.
- Intermediate Precision.
- Robustness.

SYSTEM SUITABILITY: System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.

Tailing Factor	**1.21**
Theoretical plates	**7542**
Injection No.	**Area**
1	8942634
2	8946501

3	8962584
4	8943514
5	8952588
Mean	8949564
%RSD	**0.1**

Table 2 : System suitability test of lumateperone

The tests were performed by collecting data from Single injection of blank (Diluent) and five replicate injections of Standard solution were injected into the chromatograph. The data obtained is summarized in Table

Conclusion:

- The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.

2. SPECIFICITY: (IDENTIFICATION, INTERFERENCE & PEAK PURITY)

Inject Blank (Diluent), standard solution, placebo solution and sample solution. The data obtained is summarized in Table

Solution	**Specificity data**		
	Retention time (min)	**Purity Match**	
Blank solution	NA	NA	
Placebo solution	NA	NA	
Standard solution	4.10	Purity angle	Purity threshold
		2.63	4.02
Sample solution	4.10	2.35	3.77

Table 3 : Specificity (Identification and Interference)

Sample Name: BLANK

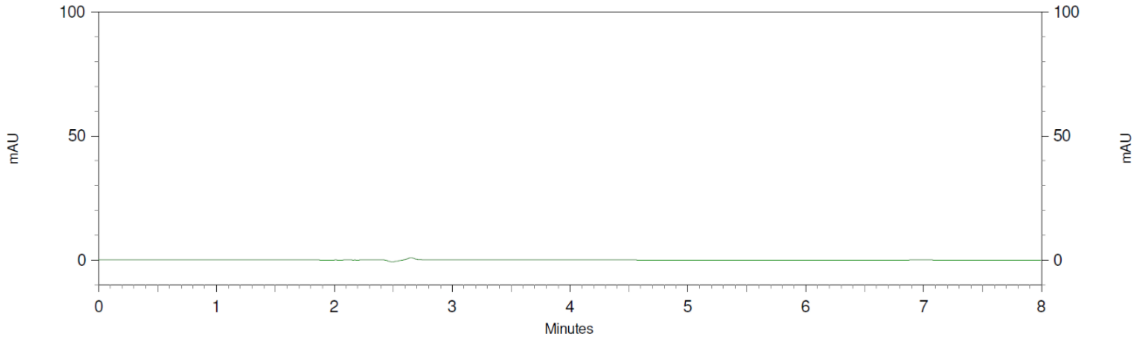

Figure 5 : Chromatogram of Blank

Sample Name: STANDARD SOLUTION_1

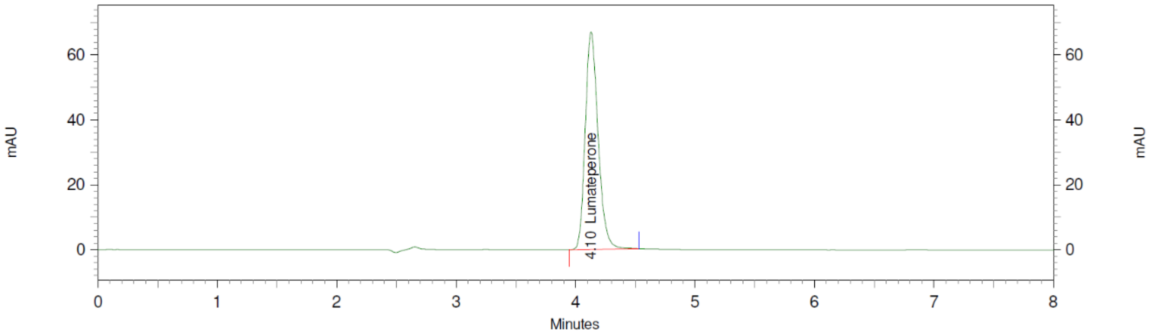

Figure 6 :Chromatogram of Standard

Sample Name: SPL

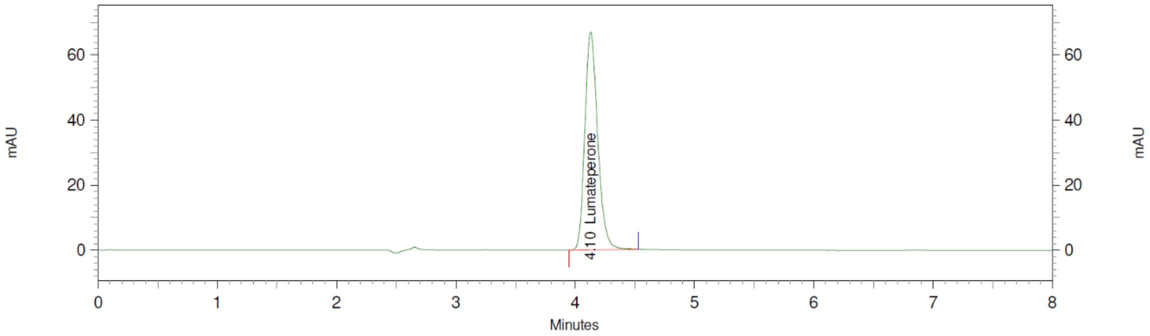

figure 7 :Chromatogram of Sample

Sample Name: PLACEBO

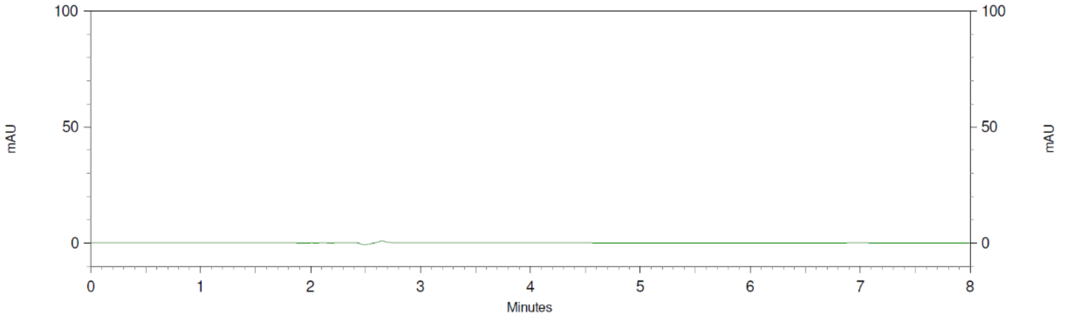

Figure 8 : Chromatogram of Placebo

Conclusion:

- The data demonstrates that retention time in standard and sample is same for Lumateperone peak.
- The data demonstrates that there is no interference in blank and placebo at the retention time of Lumateperone peak. Peak Purity match in both chromatograms obtained from Standard and Sample solution.

3. LINEARITY

Linearity was evaluated in the range of 50 % to 150 % of Lumateperone for working concentration. The working concentration of Lumateperone in solution is 50 µg/mL. The data summarized in Table.

Level	**Conc (µg/mL)**	**Area**	**Mean**
50%	25	4412539	4408405
		4416854	
		4395823	
75%	37.5	6786524	6788162
		6789701	
		6788261	
100%	50	8952419	8950737
		8949568	
		8950224	
125%	62.5	11205241	11209163
		11212684	
		11209563	
150%	75	13465201	13449324
		13426559	
		13456211	
Corr. Coeff			0.9995
Intercept			68927
Slope			177136
% Y-intercept			0.77

Table 4 : linearity plot of lumateperone

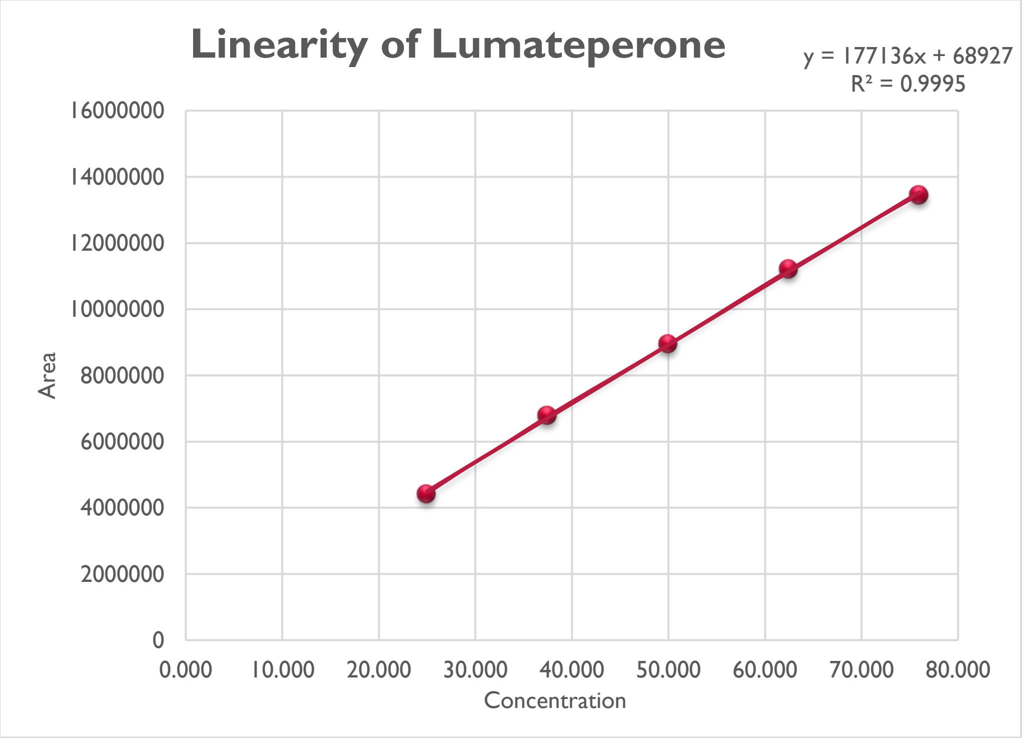

Figure 9 : Linearity plot of Lumateperone

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that the response is found to be linear.
- Co-relation coefficient (r^2 was found 0.9995.

4. ACCURACY (RECOVERY):

Evaluated accuracy from 50% to 150% of Lumateperone tablet, working concentration level. Each level prepared in triplicates.

Level (%)	**Area**	**Lumateperone Added Conc (µg/mL)**	**Lumateperone Added Conc (µg/mL)**	**% Recovery**	**Mean % Recovery**
50	4460529	25.05	24.91	99.20	99.94
	4516984	24.97	25.10	101.30	
	4490967	25.11	25.04	99.33	
100	8956109	50.02	50.02	100.00	100.02
	9026418	49.93	50.17	101.21	
	8876216	49.07	49.84	98.84	
150	13456849	74.96	75.08	100.41	99.76
	13302694	75.04	74.73	98.98	
	13412859	75.01	74.98	99.89	

Table 5 : % Recovery for Lumateperone

Conclusion:

The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 98.0% - 102.0%.

5. PRECISION

5.1 Method Precision:

Single injection of blank (Diluent), Standard solution (Five replicates) and sample solution (six preparations) was injected on the system

Sample	Area	% Assay
Sample 1	8726501	97.32
Sample 2	8820149	98.57
Sample 3	8926328	99.59
Sample 4	8702596	96.93
Sample 5	8850659	99.03
Sample 6	8741653	97.28
Mean		**98.12**
STD DEV		**1.0913**
% RSD		**1.112**

Table 6 : Method precision

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

5.2 Intermediate Precision:

six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Sample	Area	% Assay
Sample 1	8952136	99.87
Sample 2	8852149	98.22
Sample 3	8763524	98.14
Sample 4	8652363	96.57
Sample 5	8702639	97.25
Sample 6	8822642	98.10
Mean		**98.03**
STD DEV		**1.1125**
% RSD		**1.135**

Table 7: Table Intermediate Precision

Parameter	**Method Precision (Analyst-I)**	**Intermediate Precision (Analyst-II)**
HPLC NO.	**AD/HPLC-02**	**AD/HPLC-04**
Column No.	**HPLC-20**	**HPLC-26**
Sample No.	**%Assay**	
1	97.32	99.87
2	98.57	98.22
3	99.59	98.14
4	96.93	96.57
5	99.03	97.25
6	97.28	98.10
Mean	**98.12**	**98.03**
Mean of Precision % Assay	**98.07**	
Absolute Mean difference % assay	**1.1**	

Table 8: Intermediate Precision pool Data

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % Assay is of six samples is not more than 2.0
- The data shows that % Assay is within the acceptance criteria and hence the method is rugged.

6. ROBUSTNESS

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	8726501	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.3 ml/min	8856524	1.5
	1.1 ml/min	8645201	-0.9
Change in wavelength (±2 nm)	229 nm	8700416	-0.3
	225 nm	8886415	1.8

Table 9 : Robustness for Lumateperone

Conclusion:

- System suitability criteria were fulfilled.
- The difference of % assay value in each modified condition is within acceptance criteria.

7. CONCLUSION

In this study, RP-HPLC was used to measure Lumateperone in capsule form. An Agilent 1260 Infinity II HPLC system with a C18 column and UV detector was used. After testing different solvent combinations, the best mobile phase was found to be a pH 6.0 buffer and methanol. The detection was done at 227 nm, based on the UV scan results.

The method showed good separation, accurate results, and reliable performance. This confirms that RP-HPLC is a suitable and effective technique for analyzing Lumateperone in pharmaceutical capsules.

REFERENCES

[1] ICH Q2(R1). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation, 2005.

[2] FDA. Caplyta (Lumateperone) Prescribing Information.

[3] Indian Pharmacopoeia, 2022.

[4] Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed.

[5] Bakshi M, Singh S. Development of validated stability-indicating methods. J Pharm Biomed Anal. 2002;28(6):1011–1040.Rudra A, Chatterjee S, Sengupta S, Wankhede R, Nandi B, Maitra G, Mitra J. Management of obstetric hemorrhage. Middle East J Anaesthesiol 2010;20:499-507.