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Development and Validation of RP-HPLC Method for the Estimation of Lumateperone Drug in Pharmaceutical Dosage Forms.

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ABSTRACT :
This abstract outlines the development and validation of a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for quantifying lumateperone in pharmaceutical dosage forms. The study focuses on establishing a reliable analytical technique to assess the drug's concentration in formulations. Method development involves optimizing chromatographic conditions, while validation ensures the method's accuracy, precision, linearity, and robustness. The proposed RP-HPLC method proves to be a suitable tool for the routine analysis of lumateperone in pharmaceutical formulations, contributing to quality control and assurance in drug manufacturing.
Lumateperone is a medication used to manage and treat schizophrenia and other neuropsychiatric disorders. It is a second-generation atypical antipsychotic medication that exhibits a novel mechanism of action. Lumateperone's mechanism of action involves simultaneous modulation of dopaminergic, serotonergic, and glutamatergic neurotransmission. This activity describes the indications, mechanism of action, and administration of lumateperone as a valuable treatment of schizophrenia. This activity will highlight the mechanism of action, adverse effect profile, and other key factors such as dosage and interactions for the interdisciplinary healthcare team responsible for treating individuals with schizophrenia and other neuropsychiatric disorders.
KEYWORDS: RP-HPLC method, lumateperone drug, second generation atypical antipsychotic drug category.

I. INTRODUCTION :

Chromatography : Is a process for separating components of a mixture to get the process started the mixture in dissolved in a substance called the mobile phase which carries it through a second substance called the stationary phase Ex : liquid chromatography

TYPES OF CHROMATOGRAPHY : Gas chromatography, size exclusionchromatography, Ion chromatography
,Paper chromatography ,Affinity chromatography, Reverse phase High performance liquid chromatography ,Thin layer chromatography, Reverse phase High performance liquid chromatography (Rp-HPLC) involves the separation of molecule on the basis of Hydrophobicity PRINCIPLE :Separation of molecule on the basis of Hydrophobicity the separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase .

ADVANTAGES : Lower cost When compared with the other HPLC method Lower toxicity of the solvent Reduced sample evaporation

DISADVANTAGES : High cost of solvent and additives Skilled person required for operation

LUMATEPERONE DRUG –

- Lumateperone is used to treat the symptoms of schizophrenia (a mental illness that causes disturbed or unusual thinking, loss of interest in life, and strong or inappropriate emotions). Lumateperone is in a class of medications called atypical antipsychotics.
- Lumateperone is a novel 2nd generation antipsychotic used to manage both positive and negative symptoms in patients with schizophrenia.

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ADVERSE EFFECTS -

- The adverse effects associated with the administration of lumateperone are mild to moderate.
- At the FDA-approved dosage of 42mg/day, the most common side effects are somnolence, sedation, fatigue, and constipation.
- In a Phase III clinical trial consisting of 148 participants who received the currently FDA-approved dosage of 42 mg, 17.6% experienced somnolence, 12.7% experienced sedation, and 5.3% experienced fatigue. Overall, 64.7% of this group experienced adverse effects.
- No more than 5% of participants in either of the lumateperone groups (n=294) experienced extrapyramidal symptoms. Furthermore, there was no statistically significant change in median weight from the placebo.
- Metabolic endpoints, including changes in triglycerides, blood glucose, and prolactin levels, also did not change significantly from placebo; this appears to be partially attributable to its lack of affinity to off-target receptors, including histaminergic and muscarinic receptors.
- Another clinical trial compared lumateperone to a standard-of-care antipsychotic consisting of 302 patients for six weeks.
- Those who switched to lumateperone from their current antipsychotics experienced statistically significant improvements in parameters measuring LDL-cholesterol, triglycerides, and prolactin levels.
- Although there have only been a limited number of clinical trials so far, evidence points to lumateperone having a reduced risk of adverse effects normally associated with second-generation antipsychotics, although long-term studies are underway to determine the long-term safety and efficacy of lumateperone.

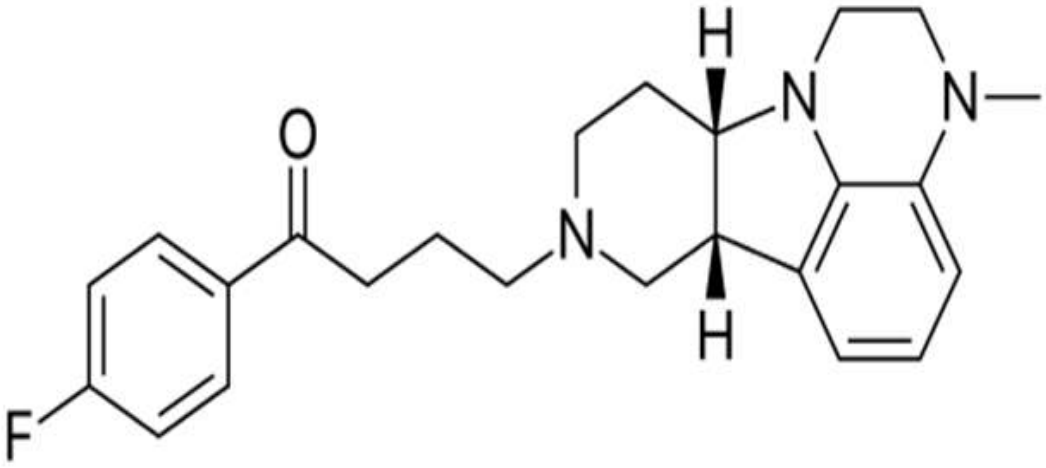

Fig : lumateperone drug

RESEARCH AIM :

The aim or the purpose of the topic "Development and Validation of RP-HPLC Method for the Estimation of Lumateperone Drug in Pharmaceutical Dosage Forms" is to establish a reliable and accurate analytical method using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) for quantifying lumateperone in pharmaceutical formulations. The development phase involves optimizing conditions for separation, while validation ensures the method's precision, accuracy, and robustness, meeting regulatory requirements for quality control in pharmaceutical manufacturing. This research contributes to the analytical methodology in pharmaceutical analysis, facilitating the quality assessment of lumateperone-containing products.

OBJECTIVES:

Develop a robust and reliable RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) method for the accurate

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estimation of lumateperone in pharmaceutical dosage forms.

Optimize chromatographic conditions to achieve high resolution, sensitivity, and specificity in the separation of lumateperone from potential impurities or excipients.

Validate the developed RP-HPLC method according to regulatory guidelines, ensuring precision, accuracy, linearity, and robustness to establish its suitability for routine analysis.

Determine the method's limit of detection (LOD) and limit of quantification (LOQ) to assess its sensitivity in detecting low concentrations of lumateperone.

Evaluate the stability-indicating nature of the method by exposing lumateperone to various stress conditions, such as heat, light, and humidity, and monitoring the chromatographic response.

Apply the validated RP-HPLC method to analyze commercially available pharmaceutical dosage forms containing lumateperone, ensuring its applicability in real-world scenarios.

Compare the results obtained with the developed method to those of a reference method or official monograph, if available, to verify its accuracy and reliability.

Assess the robustness of the method by evaluating its performance under slight variations in experimental conditions, demonstrating its consistency and suitability for routine analysis.

Document and report the entire development and validation process in a comprehensive manner, including method parameters, validation results, and any challenges encountered during the study.

Provide recommendations for the practical implementation of the validated RP-HPLC method in pharmaceutical quality control laboratories for routine analysis of lumateperone in dosage forms.

PLAN OF WORK :

Estimation of Lumateperone in capsule dosage form will be done by following methods.

Selection of Drugs and Formulation

Selection of analytical techniques

- Estimation of lambda max by UV-Visible spectroscopy.
- Development and validation of HPLC analytical method.

Method development by RP-HPLC.

- Selection of preliminary HPLC conditions.
- Selection of mobile phase.
- Selection of column.
- Selection of Flow rate.
- Selection of injection volume.
- Selection of wavelength.
- Selection of column oven temperature.
- Selection of sample Cooler temperature.
- Optimization of run time.
- Analysis of laboratory mixture.
- Validation of proposed method.
- System suitability parameter
- Linearity and Range
- Accuracy
- Precision

a. System precision.
b. Method precision.
c. Intermediate precision.

- Specificity
- Robustness

Probable outcomes:

- A simple and accurate analytical technique can be developed for the determination of Lumateperone capsule.
- Method developed can be conveniently used for quality control and routine determination of drug in pharmaceutical industry.

DRUG PROFILE :

Lumateperone:

Structure:

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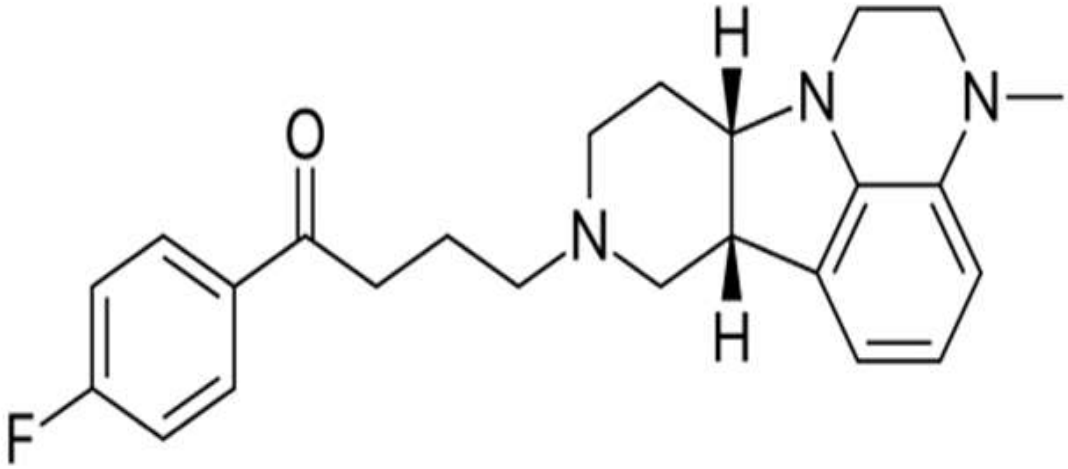

Fig: lumateperone drug

General profile of lumateperone:

- Category : Atypical antipsychotic
- Chemical Name

1(4fluorophenyl)4[(10R,15S)4methyl1,4,12Triazatetracyclo[7.6.1.0^{5,16}.0^{10,15}]hexadeca5(16),6,8-trien-12yl]butan-1-one

- Molecular Formula :C24H28FN3O
- Molecular Weight :393.506 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)

II. MATERIAL AND METHOD :

1.Materials:

Lumateperone standard (purity ≥ 99%)Pharmaceutical dosage forms containing lumateperoneHigh-performance liquid chromatography (HPLC) system with a reversed-phase (RP) columnMobile phase: Acetonitrile and water in a suitable ratioStandard laboratory equipment and glasswareMicropipettes and syringesFiltration apparatus with 0.45 µm membrane filters.

Chromatographic Conditions:

HPLC System: Use a high-quality RP-HPLC system with suitable detectors.

Column: Choose a compatible RP column (e.g., C18) with appropriate dimensions.

Mobile Phase: Prepare a mobile phase consisting of acetonitrile and water. Optimize the ratio for efficient separation.

Flow Rate: Set the flow rate to ensure good resolution and peak shape.

Detection Wavelength: Determine the optimal detection wavelength for lumateperone.

Standard Preparation:

Prepare a stock solution of lumateperone in the mobile phase.

Dilute the stock solution to obtain a series of standard solutions with different concentrations covering the expected range.

Preparation of Solution

Preparation of stock and Standard solution –

Stock solution of Lumateperone 1 mg/mL was prepared using HPLC grade methanol 42 mg of Lumateperone was dissolved in 10 mL of methanol (1 mg/mL)solution

Appropriate volumes (22-62 µg/mL of Lumateperone) of this stock solution were then further diluted with ammonium acetate to 1 mL to get the required concentrations of standard solutions at a concentration range of22-62 µg/mL.

Preparation of 0.1N NaOH weighed 4gm of NaOH was transferred in 100ml volumetric flask and diluted up to Mark with methanol. From above solution take10 ml and transferred in 100 ml volumetric flask and diluted up to the mark with methanol.

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Preparation of0.1N HCL:
Concentrated HCl(0.86ml) was transferred in 100ml volumetric flask and dilute up to the mark with methanol.

Sample Preparation:
Extract lumateperone from pharmaceutical dosage forms using a suitable extraction method.
Filter the solution through a 0.45 µm membrane filter to remove particulate matter.

Chromatographic Procedure:
Inject standard solutions and sample solutions into the HPLC system.
Record chromatograms under optimized conditions.

Selection of Wavelength:
42 µg/mL solution of Lumateperone was prepared using methanol assolvent.The above mentioned solutions were scanned Individually from 190 to 400nm in UV-Visible spectrophotometer. The optimal Response for the over plain spectrum of lumateperone was obtained at233nm.

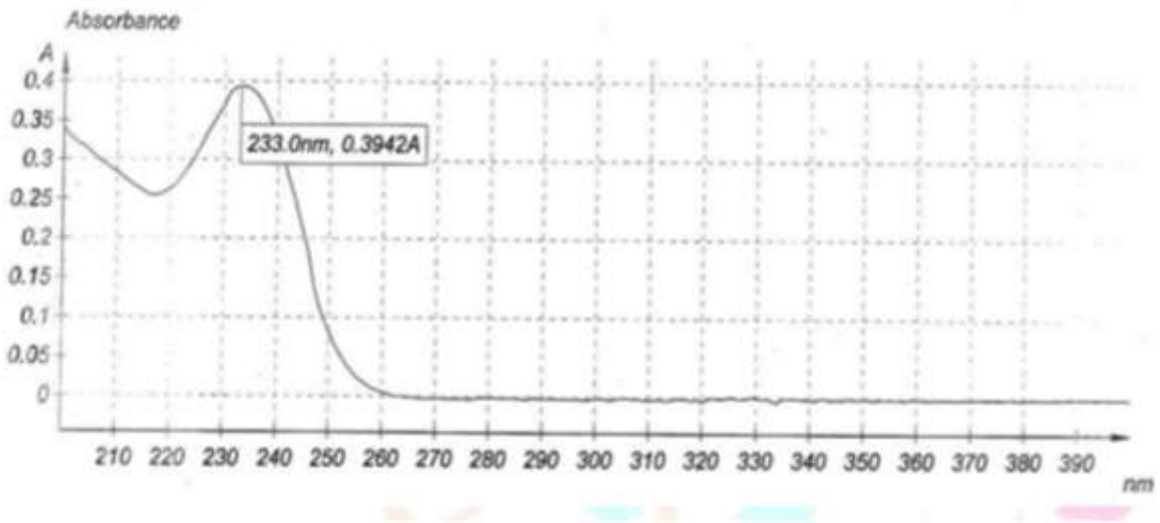

Figure : Spectra showing λ max of Lumateperone

Determination of λ max of Lumateperone

Sr no .	Wavelength(nm	Absorbance
1	233nm	0.3942A

Method development for RP HPLC and optimization.
Chromatographic conditions –

Column	Hypersil BDS C18 150 x4.6mm, 5µ
Mobile phase	Water: Methanol (90:10 v/v)
Flow rate	1.0 mL/min
Inject volume	5 µL
Wavelength	233 nm
Column Temp	26°C
Sample Temp	10°C
Run Time	60.00 minutes
Seal Wash	Methanol (90:10) v/v
Needle Wash	Water: Methanol (10:90) v/v

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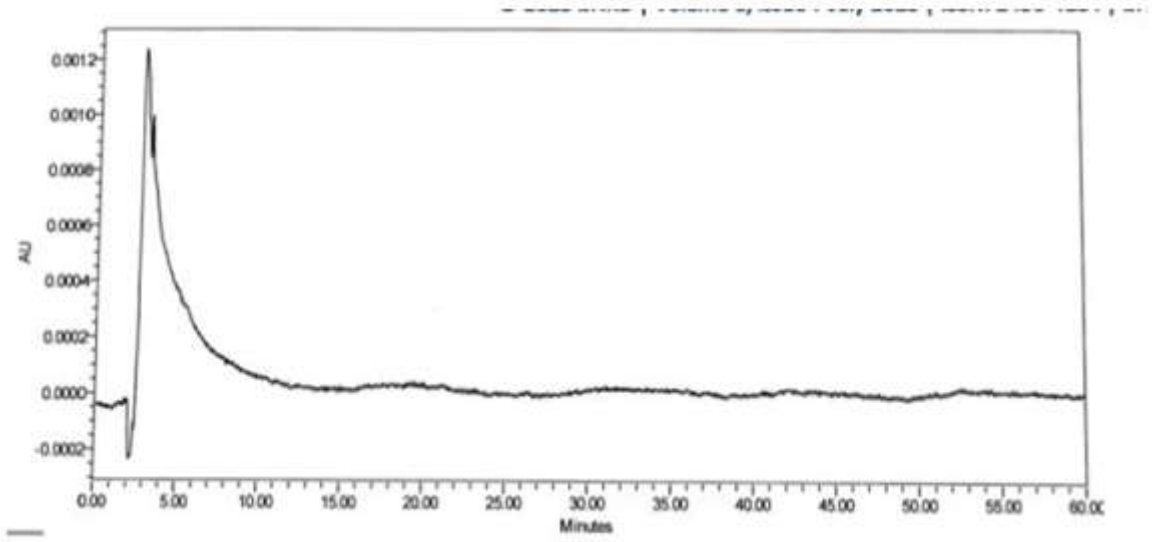

Fig. : Typical chromatogram

2.Method Validation:

Conduct validation according to ICH guidelines.

Parameters to be validated include specificity, linearity, precision, accuracy, robustness, and system suitability.

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

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

A.Specificity:

Evaluate the interference from excipients and degradation products.

Confirm the specificity of the method for lumateperone.

B.Linearity:

Construct a calibration curve by plotting peak area against concentration.

Assess linearity over a suitable concentration range.

Concentration (µg/mL)	Mean peak area with ± SD (%RSD)
22	64979± 1042.599 (0.46)
32	124765.3 ± 1054.358 (0.235)
42	189453± 9008.116 (0.061)
52	257763.3± 6810.138 (0.0287)
62	325527.3 ±8679 .238 (0.124)
REGRESSION EQUATION	y = 13082x – 3730
R	0.999
R^2	0.999

Table . Linearity data for Lumateperone (n=3)

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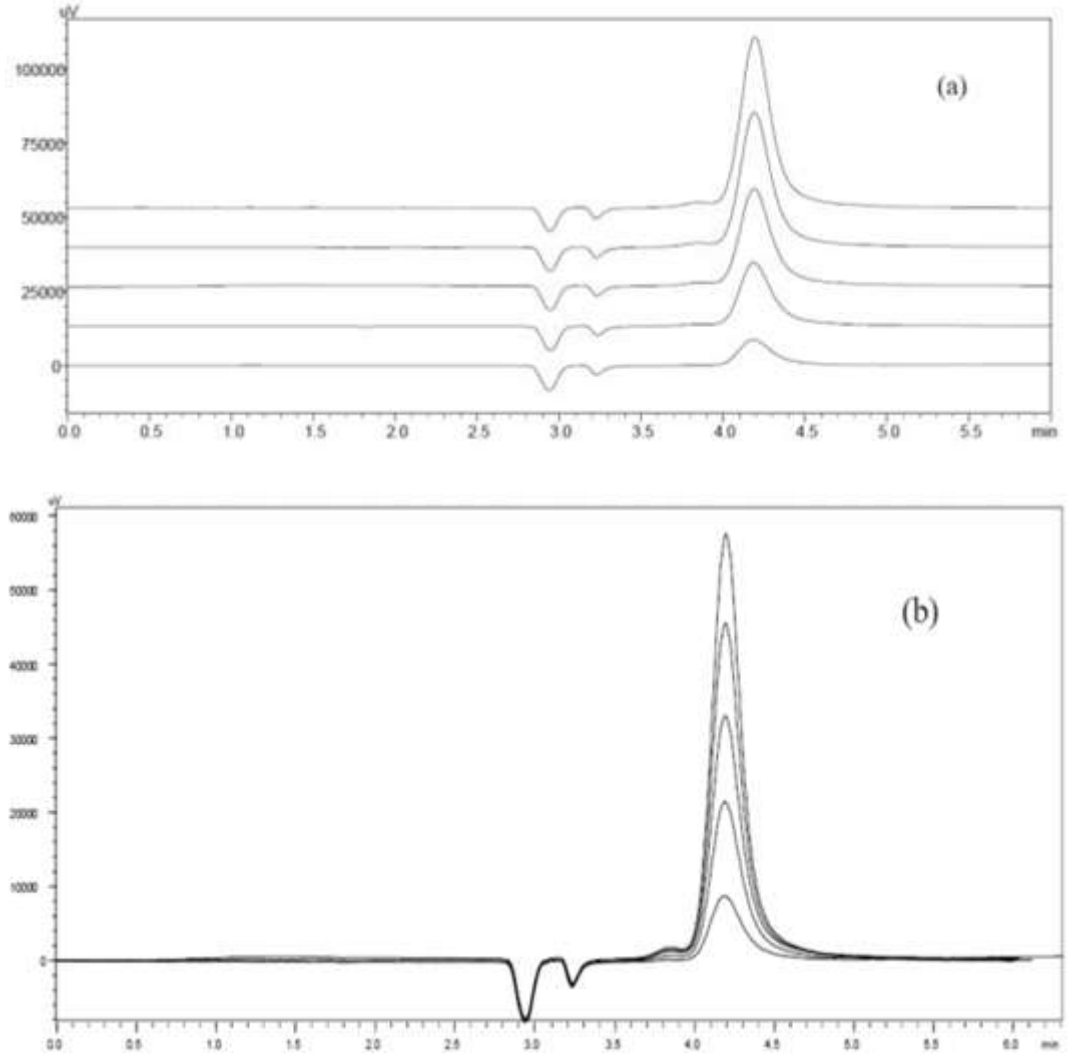

Fig.Overlay of chromatograms of standard solution of Lumateperone at different concentrations (22-62 µg/mL of Lumateperone) (a)with base shift (b)without base shift

C.Precision:

Determine intra-day and inter-day precision by analyzing replicate samples.

Calculate % RSD (relative standard deviation) for precision assessment.

D.Accuracy:

Assess accuracy by spiking known amounts of lumateperone into pre-analyzed samples.

Compare the recovered amount with the expected amount.

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Serial No.	% Level of Addition	Amount Present (µg/mL)	Amount Added (µg/mL)	Amount found (µg/mL)	% Recovery	Mean	%RSD
1	80 80 80	42 42 42	36 36 36	54.22 54.17 54.07	100.757 100.59 100.23	100.523	0.264
2	100 100 100	42 42 42	42 42 42	59.83 59.98 59.76	99.45 99.93 99.20	99.52	0.372
3	120 120 120	42 42 42	48 48 48	65.94 66.42 66.23	99.80 101.40 100.78	100.66	0.144

Table . Accuracy/Recovery data of Lumateperone

Observation :

The obtained percent recoveries and % RSD value of Lumateperone was found to be within the limits indicating the
Accuracy of the proposed method.

E.Robustness:

Evaluate the method's robustness by introducing small deliberate variations in chromatographic conditions.

Assess the impact on results and establish the method's reliability.

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.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate Variations in the method parameters and provides an indication of its reliability during normal usage. In the case of Liquid chromatography, examples of typical variations are:

- Influence of variations in mobile phase
- Influence of variations in flowrate
- Influence of variations in wavelength

As a part of the robustness, deliberate changes in the flow rate and wavelength were made to evaluate the impact on the

Method. Changes in the flowRate slightly affected the retention times of the Lumateperone. However the parameters like, theoretical plate number And tailing factor were not changed and were within the limits. Similar results were obtained with the changed Wavelength.

Change in parameter	Condition	% Assay	Absolute difference of % Assay
Control	As per method	99.3	NA
Change in flow rate1.0 ml/min(±1.0 ml/min)	0.9 ml/min	99.5	0.2
1.1ml/min		98.7	-0.6
Change in wavelength (±2 nm)	228 nm	99.8	0.5
238 nm		99.4	0.1
Change in column temperature(±5°c)	35°c	99.2	-0.1
45°c	99.3	0	

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F.System Suitability:

Verify the system suitability parameters, including resolution, tailing factor, and theoretical plates, to ensure consistent performance.

System suitability testing is an integral part of any analytical procedures. The tests are based on the concept that the

Equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system factor are

Parameters that are normally used in assessing the column performance.

System suitability studies were carried out by injecting five times standard concentration of 10µg/mL (Lumateperone)

At different injection volumes ranging from 10 L to 50 L. The RSD values for system suitability test

Parameters like retention time[Rt = 1.2560 for Lumateperone], tailing factor [Tf = 1.2018 for Lumateperone] and

Theoretical plate number [0.449 for Lumateperone] were found to be less than 2% indicating the present conditions

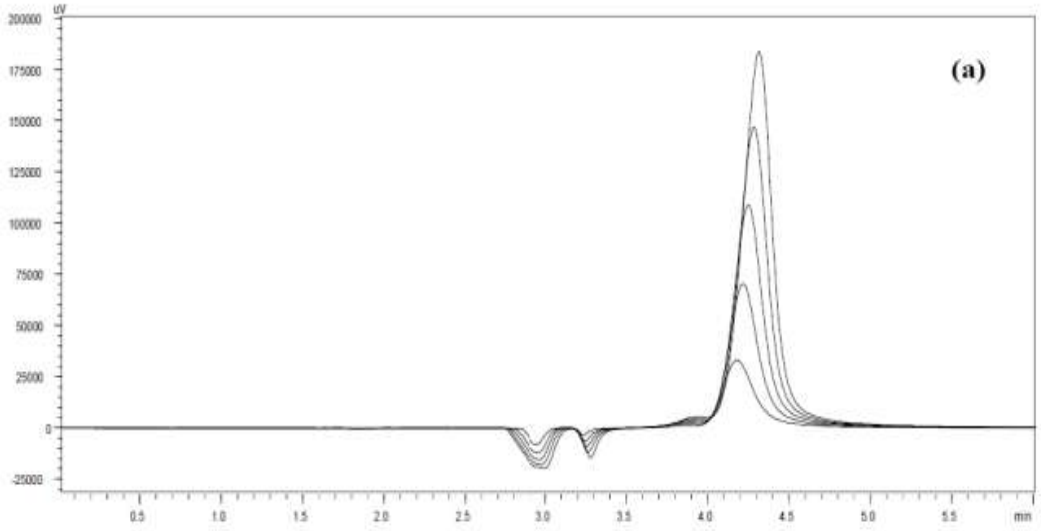
Were suitable for the analysis of Lumateperone in tablets. The data was given in Table

Acceptance Criteria

- Theoretical plate should be N>2000
- Tailing factor should beT<2

Injection Vol. (µL)	Retention time (min)	Tailing factor (Tf)	Theoretical plate (#)
22	4.175	1.426	2441.07
32	4.212	1.381	2452.19
42	4.244	1.471	2459.28
52	4.279	1.408	2471.39
62	4.310	14.12	2457.79
Mean	4.244	1.4088	2456.346
% RSD	1.2560	1.2018	0.449

Table: System suitability data for Lumateperone



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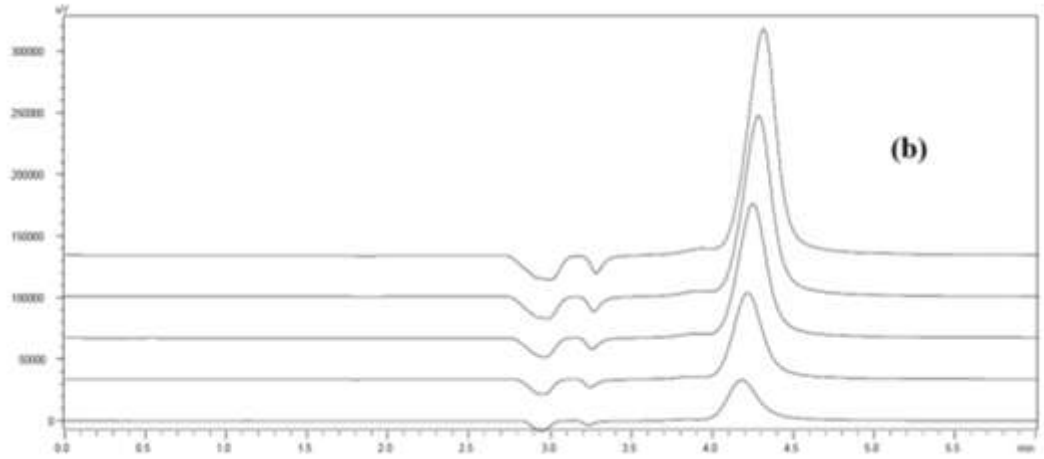

Fig. 11 Overlay {(a) without base shift (b) with base shift} of System suitability chromatograms of standard solution (22-42 µL) of Lumateperone.

Observation:
From the observation it was found that the system suitability test parameters were within limits for the proposed Method.

III. RESULT AND CONCLUSION:

Result:

The RP-HPLC method developed for the estimation of lumateperone in pharmaceutical dosage forms demonstrated excellent performance. The chromatographic conditions provided efficient separation, high resolution, and sensitivity for the target analyte. The method exhibited good linearity over a specified concentration range, with a correlation coefficient indicating a strong relationship between the concentration of lumateperone and the detector response. Precision and accuracy assessments revealed reliable and reproducible results, ensuring the robustness of the developed method.

Conclusion:

In conclusion, the developed RP-HPLC method proves to be a reliable and precise technique for the quantitative analysis of lumateperone in pharmaceutical dosage forms. The method's validation parameters, including accuracy, precision, linearity, and robustness, meet the acceptance criteria, indicating its suitability for routine analysis. This study contributes valuable analytical data that can enhance the quality control of lumateperone-containing pharmaceutical formulations, ensuring their safety and efficacy in clinical use. Further applications of the validated method could include pharmacokinetic studies and bioequivalence assessments.

QUANTITATION:

The development and validation of the RP-HPLC method for estimating lumateperone in pharmaceutical dosage forms involve establishing robust parameters for the chromatographic technique. This includes optimizing mobile phase composition, column selection, and detection wavelength. Validation encompasses assessing parameters like specificity, precision, accuracy, linearity, and robustness to ensure the method's reliability in quantifying lumateperone in various formulations.

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