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Topic : development and validation of RP-HPLC method for estimation of lumateperone drug in pharmaceutical dosage form.

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ABSTRACT

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

INTRODUCTION

In the field of pharmaceutical analysis, the development and validation of reliable and accurate analytical methods are of utmost importance. These methods play a crucial role in determining the quality, safety, and efficacy of pharmaceutical products. One such widely used technique is reversed-phase high-performance liquid chromatography (RP-HPLC). This method offers excellent separation capabilities, sensitivity, and specificity, making it an ideal choice for drug analysis.

Lumetaprone is a novel drug used in the treatment of various dermatological conditions, such as psoriasis and eczema. Its accurate determination in pharmaceutical dosage forms is essential to ensure appropriate drug dosage and therapeutic efficacy. Hence, the development and validation of an RP-HPLC method for the estimation of lumetaprone in a pharmaceutical dosage form is of great significance. The RP-HPLC method relies on the principle of differential solubility of analytes in a stationary phase (typically a hydrophobic column) and a mobile phase (solvent or solvent mixture).

The choice of column, mobile phase composition, and detection wavelength are critical factors in achieving a reliable and selective separation of lumetaprone from other components present in the dosage form. During method development, various parameters such as pH, composition, and flow rate of the mobile phase, as well as column temperature, are optimized to achieve satisfactory separation, resolution, and peak symmetry. Additionally, the selection of an appropriate internal standard, if necessary, is crucial to account for any variations during sample preparation and analysis.

OBJECTIVE:

Development and Validation of RP-HPLC Method for Estimation of Lumateperone Drug in pharmaceutical Dosage Form.

DRUG PROFILE:

lumateperone:

Structure:

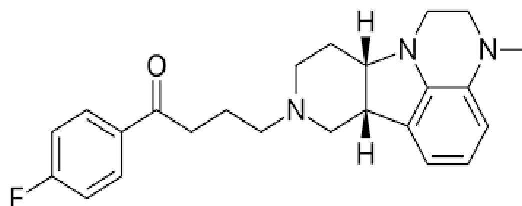

Fig. 3.1 Structure of lumateperone

Table 3.1 General profile of lumateperone

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}]hexadeca5(16),6,8-trien-12-yl]butan-1-one
Molecular Formula	C24H28FN3O
Molecular Weight	393.506 g/mole
Odou	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)

Mechanism of Action:

Lumateperone acts as a receptor antagonist of 5-HT2A receptor and antagonizes several dopamine receptors (D1, D2, and D4) with lower affinity. It has moderate serotonin transporter reuptake inhibition. It has additional off-target antagonism at alpha-1 receptors, without appreciable antimuscarinic antihistaminergic properties, limiting side effects associated with other atypical antipsychotics.

Pharmacokinetics:

After taking the medication by mouth, lumateperone reaches maximum plasma concentrations within 1–2 hours and has a terminal elimination half-life of 18 hours.[1] Lumateperone is a substrate for numerous metabolic enzymes, including various 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 cause appreciable inhibition of any common CYP450 enzymes. It is not a substrate for p-glycoprotein

PLAN OF WORK:

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

Selection of Drugs and Formulation

➢ By literature and market survey Online Journals, chemical and analytical abstracts were studied to find out drugs for which there were no more reported RP-HPLC methods. Market survey was carried to check the availability of these drugs and their dosage forms.

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.

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➢ 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. [Type the document title] 49

➢ Method developed can be conveniently used for quality control and routine determination of drug in pharmaceutical industry.

DRUG:

Name of drug and drug product	Supplier and manufacturer by
Lumateperone	Lupin Pharmaceutical Ltd.
Lumateperone Capsule 42 mg	Lupin Pharmaceutical Ltd.

REAGENTS:

Sr. No	Chemical	Make
1	Water	Rankem
2	Methanol	Merck life science
3	Potassium dihydrogen phosphate	Merck life science
4	Sodium hydroxide	Merck life science
5	Hydrochloric Acid	Merck life science
6	0.45 µ Nylon membrane disc filter	Mdi

INSTRUMENTS:

HPLC:

Sr No.	Instrument Name	Make	Model
1	Analytical balance	Mettler Toledo	XS205D0
2	Spectrophotometer	Shimadzu	UV 1900i
3	HPLC	Waters	e2695
4	PH meter	Lab India	PICO+

RESULTS AND DISCUSSION

A simple, precise and economic UV and RP-HPLC method was developed and validated for estimation of Lumateperone in capsule dosage form. The method was validated as per ICH guidelines by using various validation parameters such as Linearity, accuracy, precision, specificity and robustness.

RP-HPLC METHOD DEVELOPMENT AND OPTIMIZATION:

Selection of Wavelength

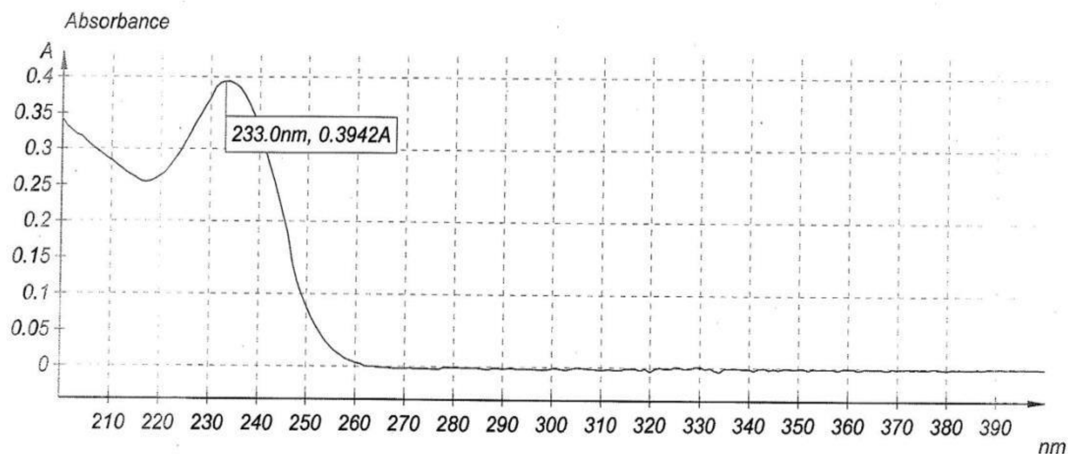


Figure : Spectra showing λ max of Lumateperone

Determination of λ max of Lumateperone

Sr. No.	Wavelength (nm)	Absorbance
1.	233	0.3942 A

Method development for RP HPLC and optimization
TRIAL: 1

Chromatographic Conditions:

Column	Hypersil BDS C18 150 x 4.6mm, 5µ
Mobile Phase	Water: Methanol (90:10 v/v)
Flow Rate	1.0 mL/min
Injection Volume	5 µL
Wavelength	233 nm
Column Temp	25ºC
Sample Temp	10ºC
Run Time	60.00 minutes
Seal Wash	Water: Methanol (90:10) v/v
Needle Wash	Water: Methanol (10:90) v/v

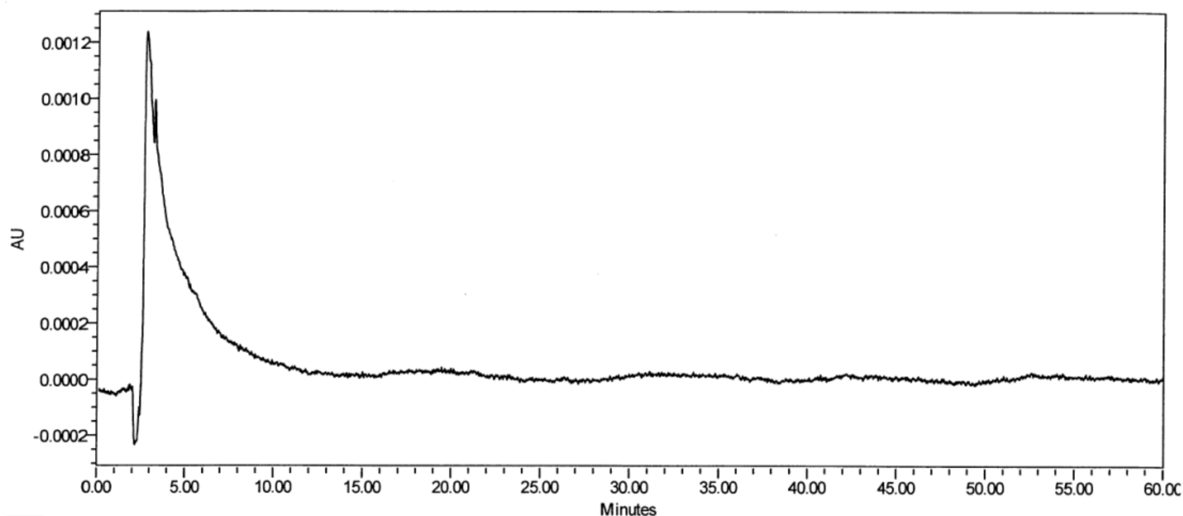


Fig. : Typical chromatogram for Trial- 1

Conclusion:
Lumateperone peak not observed at chromatogram, peak not eluted hence Method need to optimize
TRIAL: 2

Column	Hypersil BDS C18 150 x 4.6mm, 5µ
Mobile Phase	water: Methanol (60:40 v/v)
Flow Rate	1.0 mL/min
Injection Volume	5 µL
Wavelength	233 nm
Column Temp	25ºC
Sample Temp	10ºC
Run Time	7.00 minutes
Seal Wash	Water: Methanol (90:10) v/v
Needle Wash	Water: Methanol (10:90) v/v

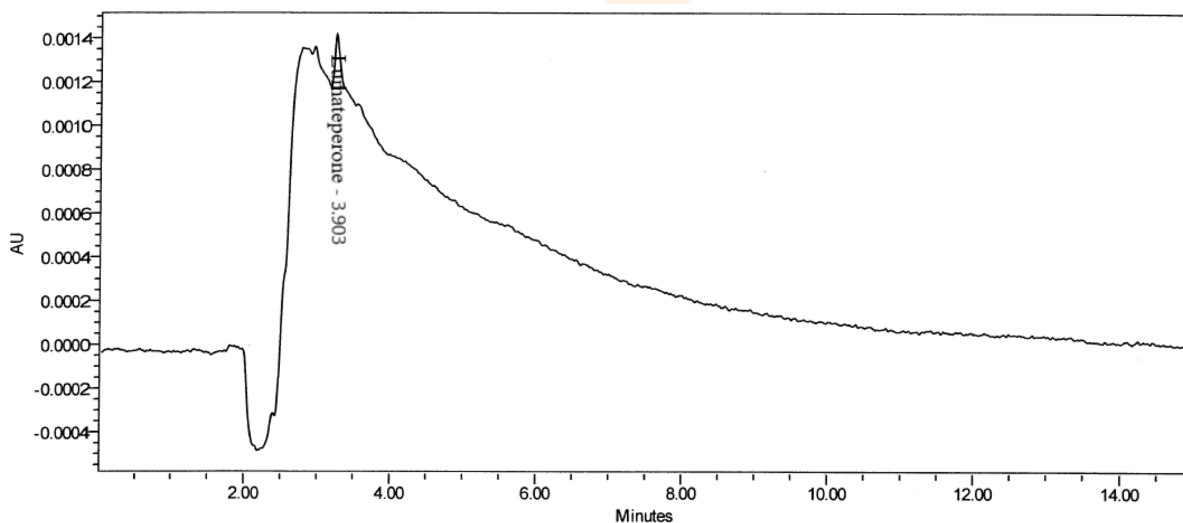


Fig. : Typical chromatogram for Trial- 2

Conclusion:
Lumateperone peak observed at 3.9 min in chromatogram, peak eluated in baseline pattern. hence stationary phase and mobile phase need to optimize

TRIAL: 3 Chromatographic Condition

Column	Zodiac C18, 150 x 4.6mm, 5µ
Mobile Phase	Buffer pH 6.2: Methanol (60:40 v/v)
Flow Rate	1.0 mL/min
Injection Volume	5 µL
Wavelength	233 nm
Column Temp	40°C
Sample Temp	10°C
Run Time	7.00 minutes
Seal Wash	Water: Methanol (90:10) v/v
Needle Wash	Water: Methanol (10:90) v/v

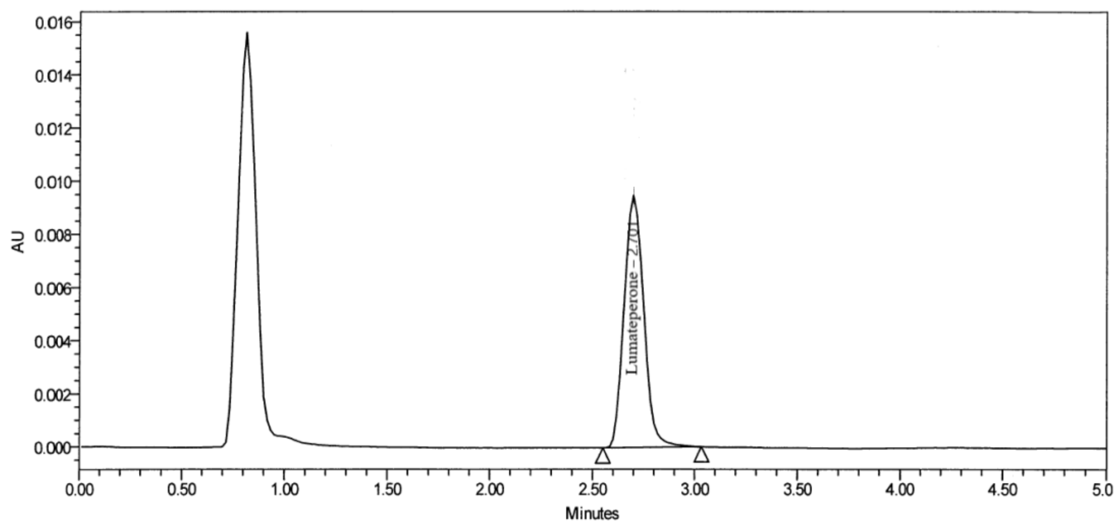


Fig. : Typical chromatogram for Trial- 3

Conclusion:- Lumateperone peak observed 2.701 min at chromatogram, peak not symmetric, peak response need to increase. hence method need to optimize

TRIAL: 4 Chromatographic Condition

Column	Zodiac C18, 150 x 4.6mm, 5µ
Mobile Phase	Buffer pH 6.2: Methanol (80:20 v/v)
Flow Rate	1.0 mL/min
Injection Volume	10 µL
Wavelength	233 nm
Column Temp	40°C
Sample Temp	10°C
Run Time	7.00 minutes
Seal Wash	Water: Methanol (90:10) v/v
Needle Wash	Water: Methanol (10:90) v/v

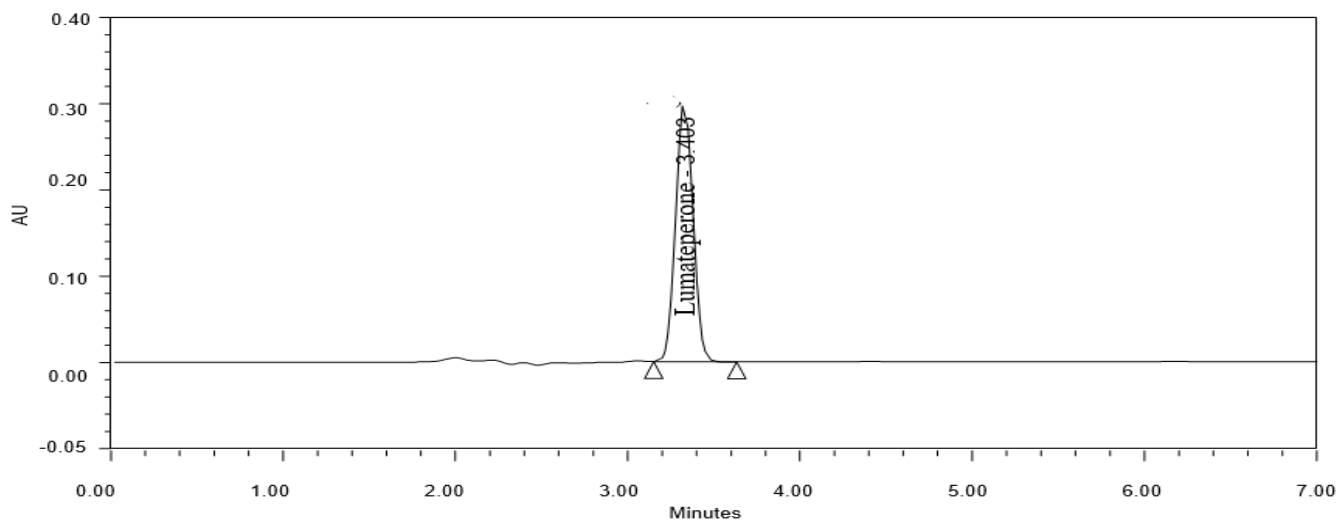


Fig. : Typical chromatogram for Trial- 4

Conclusion:- Lumateperone peak observed at 3.403 min. Peak showing all system suitability parameter. Hence method is specific accurate precise for further use.

METHOD VALIDATION

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.

system suitability test of Lumateperone

Tailing factor	**1.0**
Theoretical plates	**10245**
Injection No.	**Area**
1	171347
2	171614
3	170565
4	169478
5	170624
6	170402
Mean	170672
%RSD	0.4

Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution, impurity Solution, placebo solution and sample solution.

Solution		**Specificity data**	
Retention time		**Purity Match**	
Blank solution		NA	NA
Placebo solution		NA	NA
3.328		Purity angle	Purity threshold
Standard solution		0.68	1.36
Sample solution	3.384	0.53	1.21

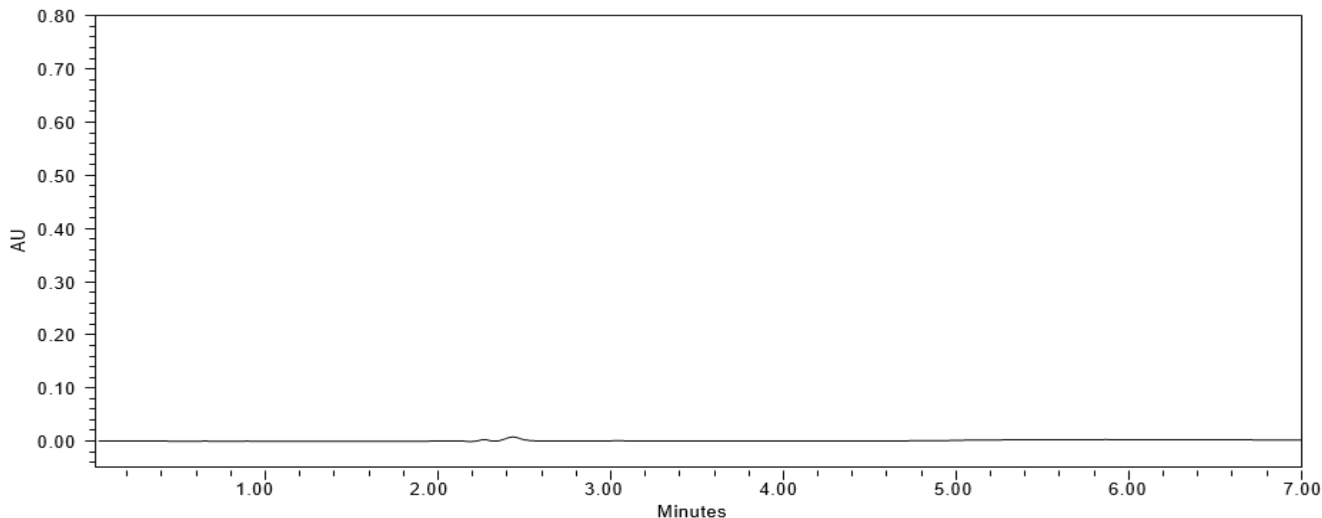


Fig : Chromatogram of Blank

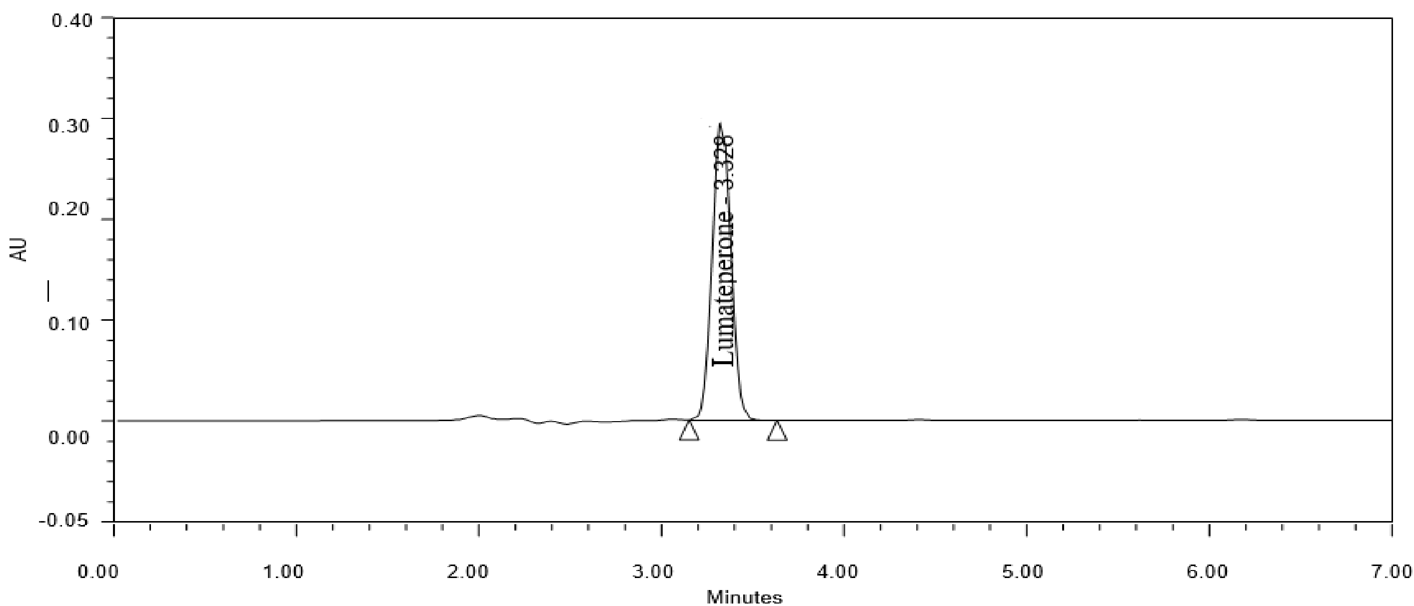


Fig : Chromatogram of Standard

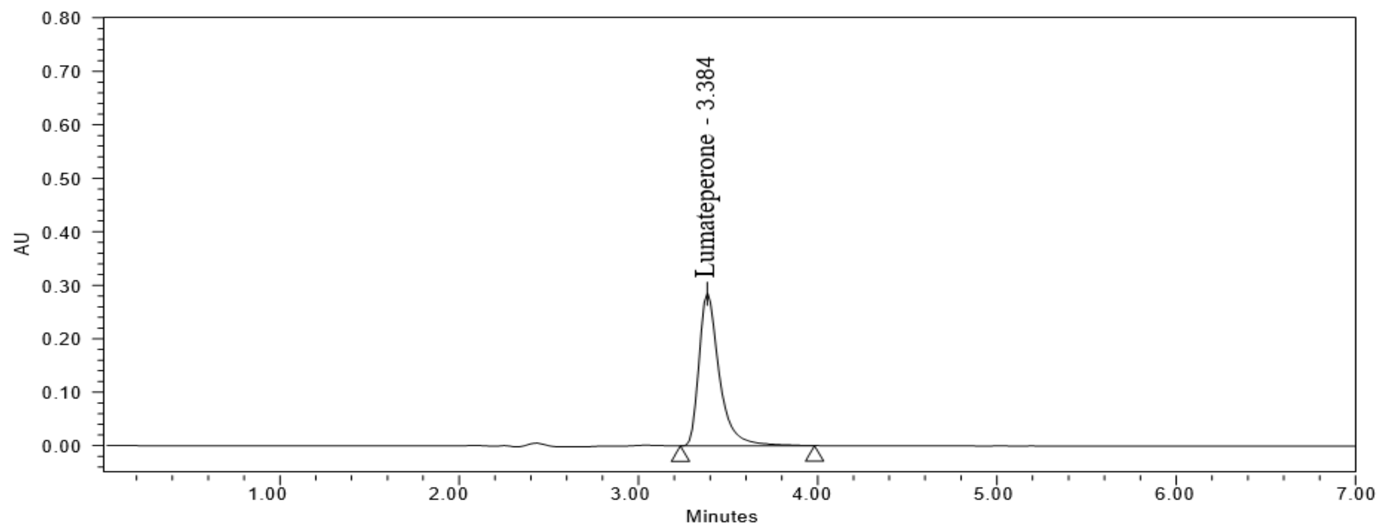


Fig : Chromatogram of Sample

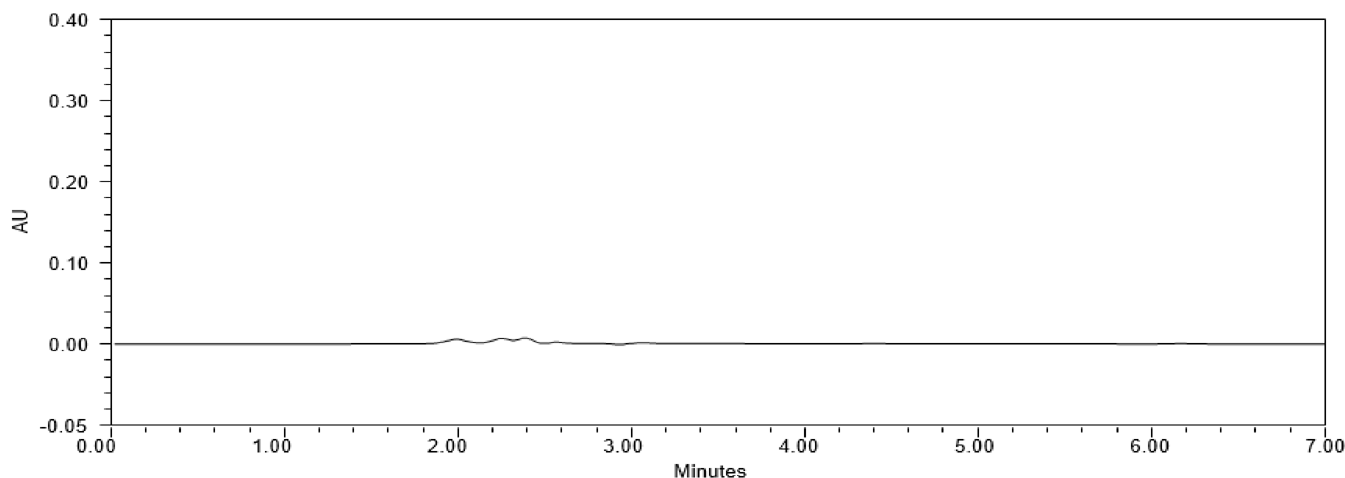
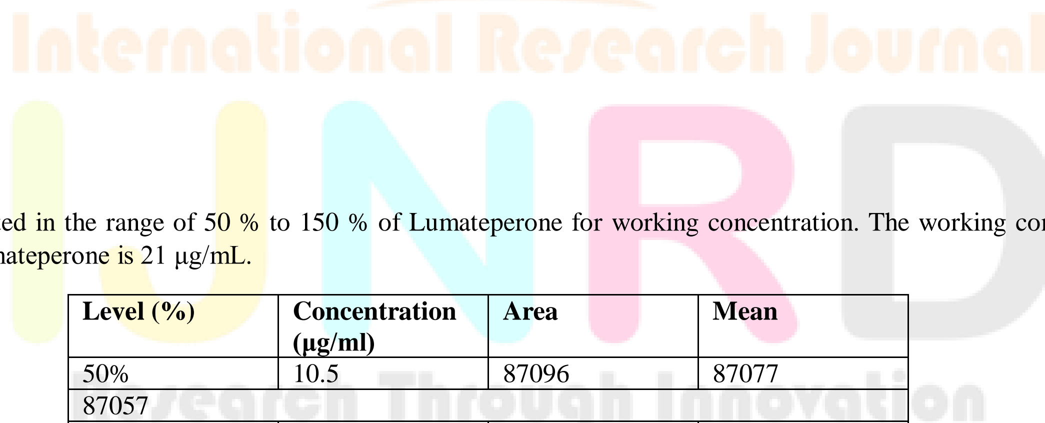


Fig : Chromatogram of Placebo



LINEARITY:

Linearity was evaluated in the range of 50 % to 150 % of Lumateperone for working concentration. The working concentration of Lumateperone in Lumateperone is 21 µg/mL.

Level (%)	**Concentration (µg/ml)**	**Area**	**Mean**
50%	10.5	87096	87077
87057			
75%	15.75	131452	131228
131004			
100%	21	175234	174900
174565			
125%	26.25	218149	218553
218957			
150%	31.5	260700	260745
260789			
Correlation Co-coefficient(r)		0.9999	
Intercept		8279.6	
Slope of regression line		636.2	
%Y- intercept			

% Level	Conc.	Area	Mean Area	% Recovery	Mean
50 %	10.5	86104	86053	100.7	100.7
86002					
10.5	84512	84522	99.0		
84532					
10.5	84968	85047	99.7		
85126					
100%	169540	169043	99.0	99.0	
168546					
171563	171293	100.4			
171023					
170005	170603	99.9			
171204					
254563	254536	99.4	99.4		
254512					
150%	255684	256570	100.2		
257456					
257654	257306	100.5			
256958					

Precision:

7.2.5.1 System Precision:

Single injection of Blank (Diluent) and six replicate injections of Standard solution were injected into the chromatographic system

Sr. No.	**Area**
1	172300
2	170259
3	169649
4	171627
5	170809
6	170504
Mean	170858
%RSD	**0.6**

Method Precision:

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

Sample set	**Area**	**% Assay**
Set-1	170858	99.6
Set-2	169584	98.9
Set-3	168954	98.5
Set-4	170245	99.2
Set-5	171000	99.7
Set-6	169586	98.9
Mean	**99.1**	
% RSD	**0.5**	

Intermediate Precision:

Parameter	**Method Precision (Analyst-I)**	**Intermediate Precision (Analyst-II)**
HPLC NO.	**HPLC-02**	**HPLC-04**
Column No.	**HC-43**	**HC-68**
Sample No.	**%Assay**	
1	99.6	98.6
2	98.9	99.4
3	98.5	98.8
4	99.2	98.5

5	99.7	99.5
6	98.9	98.5
Mean	99.1	99.0
Absolute Mean difference % assay	**0.4**	

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	% 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	

CONCLUSION
9.1 RP-High Performance Liquid Chromatography (HPLC) Method:
HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique was employed in the present investigation for estimation of Lumateperone Capsule formulation. HPLC Water2469 with GL-Science, Zodiac C18, 150 x 4.6mm, 5µ column and UV/PDA detector with empower pro Software was used for the study. The standard and sample solution of Lumateperone Capsule were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.
During selection and optimization of the mobile phase it was observed that the sharpness of the peak is achieved in Buffer pH 6.2: Methanol (80:20 v/v).
The mobile phase that was found to be most suitable was water and methanol, the wavelength 233 nm were selected for the evaluation of the chromatogram of Trimipramine maleate Capsule respectively. The selection of the wavelength was based on the λ max obtained by scanning of standard laboratory mixture in water: Methanol. This system gave good resolution and optimum retention time with appropriate tailing factor (<2).
After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by procedure described under Materials and methods. It gave accurate, reliable results and was extended for estimation of drugs in oral formulation.
The results from table clearly indicate that the RP-HPLC technique can be successfully applied for the estimation of above-mentioned drugs in their formulation.

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