Atomic Spectroscopy

Article

Contemporary HPLC-UV reverse phase method development and validation of anti-psychotic drug Lumateperone Tosylate, followed by forced degradation studies

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ABSTRACT: Background and Objective: Lumateperone an atypical anti-psychotic drug, approved for treatment of schizophrenia and bipolar depression. The present study is aimed for the development and validation of RP-HPLC method, the detection of Lumateperone followed by forced degradation studies.
Materials and Methods: Lumateperone is separated on a Kromasil C18, 150x 4.6mm, 5µm, 180Ao column using an isocratic mobile phase consisting of 0.01N potassium dihydrogen ortho phosphate and acetonitrile in the ratio of 60:40 at 30°C. The chromatographic detection was done using UV-VIS spectrophotometer at 245.0 nm. The developed method was validated for accuracy, precision, selectivity, linearity, solution stability, LOD and LOQ as per ICH guidelines.
Results: The method precision was found to be 99.78% with an RSD of 0.68%. The established LOD and LOQ are 0.22 and 0.66 µg/mL respectively. Oxidation of drug with 20% hydrogen peroxide (H2O2), acid degradation with 2N hydrochloric acid, alkali degradation using 2N sodium hydroxide, dry heat degradation, neutral degradation, and photo stability studies were evaluated as part of degradation studies with %recovery more than 94.4%.
Conclusion: This study was out with an explicit, clear and precise method and validated for all the parameters as per ICH guidelines with a high percentage recovery under various conditions of degradation

Keywords: Lumateperone, Antipsychotics, Schizophrenia, HPLC, Method Validation, Forced Degradation.

INTRODUCTION

The term antipsychotics was known to be antischizophrenic drugs used for treating schizophrenia, which is the most common forms of mental illness. Antipsychotics are also termed as neuroleptics and major tranquillizers [1]. Dopamine and glutamate are the main neurotransmitters involved in the pathogenesis of schizophrenia. It is characterized by psychotic illness which is characterised by delusions, hallucinations, and a positive symptom to thought disorder, social withdrawal symptoms and negative symptoms of flattening of emotional responses, cognitive impairment is also seen [2, 3]. Frequent recurring of acute episodes with positive symptoms may develop chronic schizophrenia with negative symptoms. Antipsychotic drugs are broadly classified in to first generation antipsychotics also called as typical antipsychotics or conventional antipsychotics or classical antipsychotics and second-generation antipsychotics are called as atypical antipsychotics. The two generation drugs are distinguished in receptor profile, efficacy, and incidents based on extrapyramidal side effects [4, 5].

Lumateperone is a second-generation antipsychotic drug is approved by USFDA on December 20, 2019. Its chemical name is 1-(4-Fluorophenyl)-4-(3-methyl-2,3,6b,9,10,10a-hexahydro- 1H-pyrido[3',4':4,5]pyrrolo[1,2,3-de]quinoxalin-8(7H)-yl)-1-butanone with molecular formula: $C_{24}H_{28}FN_3O$ [5, 6]. Currently it is in clinical trials, in development studies for bipolar depression and other neurological indications. Pharmacologically, Lumateperone acts as 5-HT 2A receptor antagonist and it also antagonizes the action of several dopamine receptor subtypes namely D1, D2, D4. It also acts as a serotonin reuptake inhibitor, but action is moderately seen. It has mild antimuscarinic activity [7, 8, 9].

Current study aims to modernize reverse phase HPLC method for the estimation of Lumateperone in pharmaceutical dosage forms and to validate it. The study also aims to find out chemical behaviour of Lumateperone when exposed to different forced degradation conditions and to identify their effect on purity of Lumateperone.

MATERIALS AND METHODS

Chemicals, reagents, and instruments

The Lumateperone standard was purchased from BMR chemicals, Hyderabad with purity more than 98%. Water, acetonitrile, methanol of HPLC grade were used and other chemicals like orthophosphoric acid (88%), glacial acetic acid, triethyl amine, dimethyl sulfoxide, perchloric acid, potassium di hydrogen orthophosphate, sodium hydroxide, sodium

phosphate dibasic anhydrous, sodium acetate, ammonium acetate, di-Sodium hydrogen orthophosphate were of analytical grade and purchased from RANKEM chemicals. Waters make HPLC (2695 model) with PDA detector (2996 model) was used.

Preparation of reagents
Preparation of 0.01N potassium dihydrogen ortho phosphate buffer solution (pH 4.8):
1.36gm of potassium dihydrogen ortho phosphate was taken in to a 1000ml volumetric flask and 900mL of HPLC grade water was added and sonicated well. Later the volume was made up to 1000mL with same diluent and finally the pH of the solution was adjusted to 4.8 with glacial acetic acid.

Preparation of mobile phase
0.01N Potassium dihydrogen ortho phosphate buffer (pH 4.8) and were taken in the ratio 60:40.

Preparation of diluent: Transferred 50mL of 0.1% ortho phosphoric acid in water to a 100mL reagent bottle and added 50mL of acetonitrile, mixed the contents and sonicated for 2 minutes.

Preparation of Solutions
Preparation of standard solution
Accurately weighed 42mg of Lumateperone Tosylate standard and transferred into a 100 mL volumetric flask, dissolved with 50mL of diluent and sonicated for 2 minutes. Finally made up the volume with diluent.

Preparation of working standard solution
1mL of above standard solution was diluted to 10 mL with diluent in a volumetric flask to produce 42µg/mL of Lumateperone working standard solution.

Preparation of sample stock solution
Equivalent weight of the combination powder sample of Lumateperone was accurately weighed and transferred into a 100mL volumetric flask and dissolved initially with 50 mL of diluent and sonicated for 2 minutes. Finally made up the volume with diluent and filtered.

Preparation of working sample solution
1mL of above Sample stock solution was diluted to 10 mL with diluent in a volumetric flask to produce produce 42µg/mL of Lumateperone sample solution.

Preparation of Solutions for Forced Degradation Studies
Oxidation
1mL of Lumateperone standard solution was taken and to it 1mL of 20% hydrogen peroxide was added. Solution was kept aside for 30 min. at 60˚C. The resulting solution was diluted to obtain the 42µg/mL concentration solution and 10µL of the sample was injected into HPLC system for the assessment of stability of the sample towards oxidation.

Acid degradation
1mL of Lumateperone standard solution was taken and to it 1mL of 2N hydrochloric acid solution was added and refluxed for 30 min. at 60˚C. The resulting solution was diluted to obtain 42µg/mL concentration solution and 10µL of the sample was injected into HPLC system for the assessment of stability towards acid.

Alkali degradation
1mL of Lumateperone standard solution was taken and to it 1mL of 2N sodium hydroxide solution was added and refluxed for 30 min. at 60˚C. The resulting solution was diluted to obtain 42µg/mL concentration solution and 10µL of the sample was injected into HPLC system for the assessment of stability towards alkali.

Dry heat degradation
Lumateperone standard solution was taken and placed in an oven at 105˚C for 6hrs for dry heat degradation studies. After the elapsed period, the solution was diluted to obtain 42µg/mL concentration solution and 10µL of the sample was injected into HPLC system for the assessment of stability.

Photostability
The drug stability towards photochemical degradation was studied by exposing the standard solution to UV light under UV chamber for 7 days or 200-watt hours/min. The resulting solution was diluted to obtain 42µg/mL concentration solution and 10µL of the sample was injected into HPLC system for the assessment of stability.

Neutral degradation studies
Under neutral conditions stress testing was done by refluxing drug in water for 6hrs at a temperature of 60˚C. The resulting solution was diluted to obtain 42µg/mL concentration solution and 10µL of the sample was injected into HPLC system for the assessment of stability.

HPLC optimized method

Considering the nature and solubility characteristics of Lumateperone, HPLC with reverse phase was better choice of analysis. A series of experiments were performed by making few changes in the solvent system, ratio of mobile phase and composition and the flow rate. Under isocratic conditions, achieved ideal separation for Lumateperone on an Kromasil C_{18} 150x 4.6mm, 5µm 180A° column with a mobile phase consisting of 0.01N Potassium dihydrogen ortho phosphate and acetonitrile in the ratio of 60:40 at 30 °C. UV-VIS spectrophotometric detection was done at 245.0 nm. These conditions were found suitable for the separation with good drug recovery. The chromatographic conditions were given in Table 1 and representative chromatogram in Fig 1.

The method developed was validated for, accuracy, precision, selectivity, linearity, solution stability and solubility at 24hrs, LOD and LOQ as per ICH guidelines. forced degradation studies were also performed to establish drug degradation pathways which reveals chemical behaviour of Lumateperone and samples obtained from degradation studies were analysed. The degradation parameters include oxidation of drug with 20% hydrogen peroxide (H_2O_2), acid degradation using 2N hydrochloric acid, alkali degradation with 2 N sodium hydroxide, dry heat degradation, neutral degradation, and photostability studies.

RESULTS AND DISCUSSION

Developed method was validated for all the parameters according to ICH guidelines. Sample injections were repeated five times and the purity of drug estimation which means assay was estimated using standard calibration curve method.

Validation of proposed method

Linearity

Six injections were eluted with 25, 50, 75, 100, 125, 150 % of the linearity levels and repeated for six sets. The average area found for each level was given in the table. Linearity was constructed and the slope was found to be 34625 with y- intercept 2192.5 giving out the correlation coefficient 0.9995. The %linearity, concentration of drug, area of the peaks were given in Table 2 and linearity graph was given in Fig 2.

Method Precision or Repeatability

Working sample solution are injected six times and eluted for the repeatability of the solution at a concentration of 42 µg/ml. The average peak area of the solutions, standard deviation, %RSD values are given in the Table 3.

Day To Day Precision or Intermediate Precision

Working Sample solution are injected six times next day of the preparation of the solution at 24 hrs of intermediate precision at a concentration of 42 µg/mL to perform intermediate precision. The average peak area of the solutions, standard deviation, %RSD values are given Table 4 and chromatogram after 24hrs of intermediate precision was given in Figure 2.

Limit of detection and limit of Quantification

Drug concentrations of range 2.5 to 12.5 µg/mL which is expected detection limit range. Calibration curve was drawn using these concentrations. Y-intercept of regression line, standard deviation was calculated and submitted in the following equations to find out the limit of detection and limit of quantification of the drug. The values were given in Table 5.

Limit of detection = $\sigma \times 3.3/S$

Limit of quantification = $\sigma \times 10/S$

σ = Standard deviation

S = Slope

Accuracy

Three concentrations levels for accuracy of 50%, 100%, 150% were prepared and each level of concentration were injected three times and percentage recovery, standard deviation was calculated. The percentage recovery at each level of accuracy and the standard deviation and relative standard deviation ns data is shown in Table 6.

System Suitability

working standard solution of Lumateperone was prepared as per the procedure and six injections were given for attaining system suitability parameters. Area of the peak, USP plate count and the tailing factor were calculated and shown in the Table 7.

Robustness

To achieve robustness, small changes in the chromatographic conditions were employed. These changes were applied according to ICH guidelines and the change in flowrate, mobile phase composition, and temperature were applied and injected, %RSD was found within limits specified in Table 8.

Forced Degradation Studies

Acid Degradation studies

Degradation in acid was performed and after study it was found that there is no degradation in the drug and good drug recovery, and theoretical plates. Purity plot was also given for the acid study. The chromatograms of the acid degradation study and the purity plot are given in Fig 3 and Fig 4.

Alkali degradation studies

Degradation in base was performed and after study it was found that there is no degradation in the drug. good drug recovery, and theoretical plates was achieved. Purity plot was also given for the alkaline study. The chromatograms of the base degradation study and the purity plot are given in Fig 5 and Fig 6.

Peroxide degradation studies
Peroxide degradation was performed and no degradation in the drug was seen. good drug recovery, and theoretical plates was achieved. Purity plot was also drawn for peroxide degradation study. The chromatograms of study and the purity plot are given Fig 7 and Fig 8.

Photolytic degradation study
Degradation in ultra-violet was performed and after study it was found that there is no degradation in the drug. good drug recovery, and theoretical plates was achieved. Purity plot was also given for the study. The chromatograms and the purity plot are given in Fig 9 and Fig 10.

Neutral degradation study
Degradation in water was performed and after study it was found that there is no degradation in the drug. good drug recovery, and theoretical plates was achieved. The chromatograms of the base degradation study and the purity plot are given in Fig 11 and Fig 12.

CONCLUSION

This method was developed for the determination of Lumateperone separated on an Kromasil C_{18} 150x 4.6mm, 5µm 180A° column by isocratic elution with a mobile phase consisting of 0.01N Potassium dihydrogen ortho phosphate and acetonitrile at 30 °C in the ratio 60:40. Detection was done by using UV-VIS spectrophotometric detector at 245.0 nm. Method developed was entirely validated for, accuracy, precision, selectivity, linearity, solution stability and solubility at 24hrs, LOD and LOQ as per ICH guidelines. Forced degradation studies were also performed to establish drug degradation pathways which reveals chemical behaviour of Lumateperone and samples obtained from degradation studies were analysed. Method precision for the developed method was found to be 99.78% with 0.68%RSD, with LOD and LOQ at 0.22 and 0.66 µg/mL respectively. Degradation parameters include oxidation of drug with 20% hydrogen peroxide (H_2O_2), acid degradation using 2N hydrochloric acid, alkali degradation with 2 N sodium hydroxide, dry heat degradation, neutral degradation, and photo stability studies were performed and the %recovery was not less than 94.4%. This study was out with a explicit, clear, and precise method and validated for all the parameters as per ICH guidelines with a high percentage recovery under various conditions of degradation. This method is best suitable for routine analysis for the estimation of Lumateperone in pharmaceutical dosage forms.

Financial support and sponsorship
Nil.

Conflicts of Interest:
There are no conflicts of interest.

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HPLC optimized method:

Table 1: chromatographic conditions of optimized method

Flow rate	1ml/min
Column	Kromasil 150x 4.6mm, 5µ.
Detector wavelength	245.0 nm
Column temperature	30°C
Injection volume	10.0µL
Run time	4.0 minutes

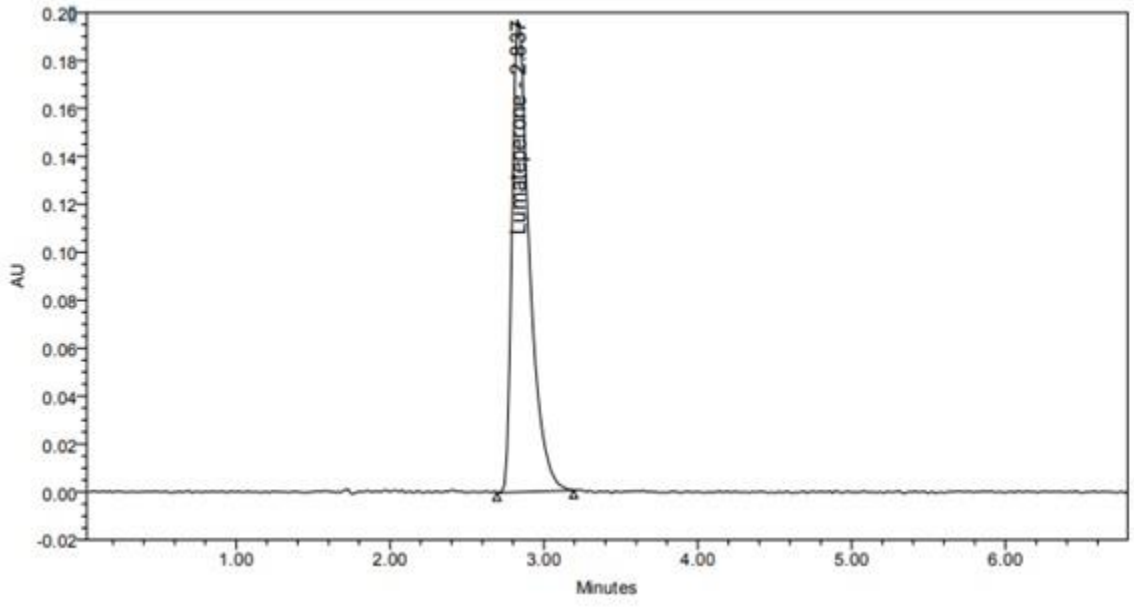

Figure 1: Chromatogram of Lumateperone

Linearity:

Table 2: Linearity results of Lumateperone

% of Linearity Level	Concentration of drug	Area of the peak
0	0	0
25	10.5	351740
50	21	724977
75	31.5	1131195
100	42	1447213
125	52.5	1824075
150	63	2170938

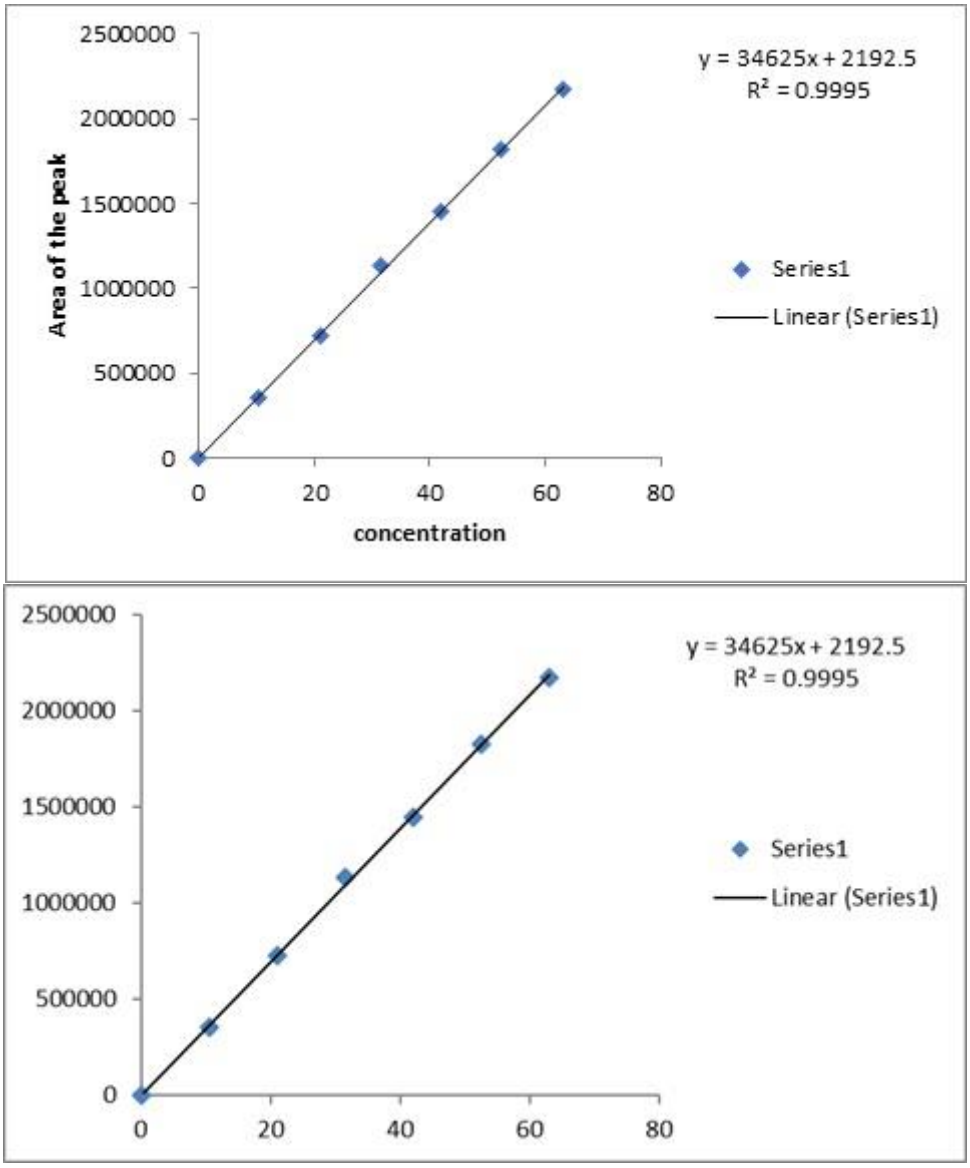

Figure 2: Linearity plot

Method Precision or Repeatability:

Table 3: Method precision data

Serial number of injections	Area of the peak
1	1434925
2	1429383
3	1423240
4	1443206
5	**1422143**
6	1444940
AVG	1432973
SD	9763.6

% RSD	0.7

Day To Day Precision or Intermediate Precision:

Table 4: Intermediate precision data

Serial number of injections	Area of the peak
1	1415109
2	1389403
3	1386227
4	1411730
5	1391820
6	1387622
AVG	1396985
SD	12910.9
% RSD	0.9

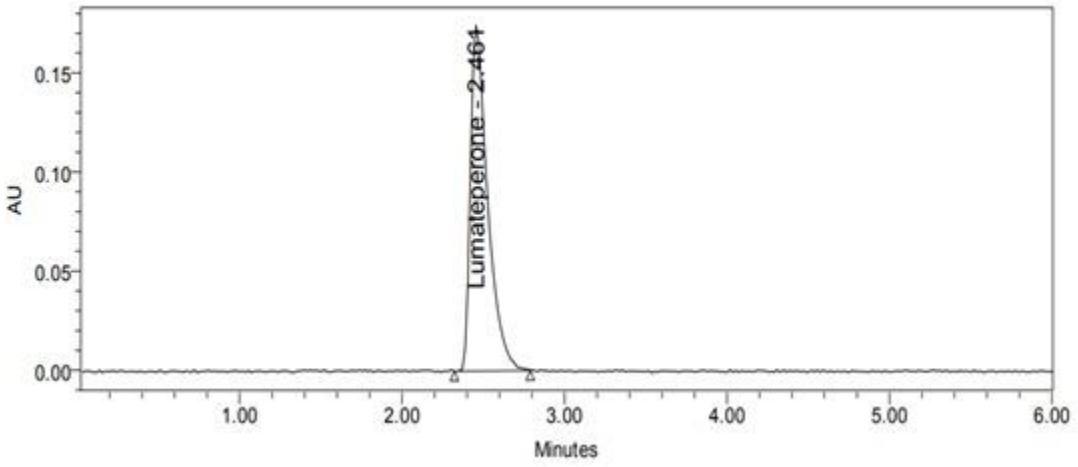

Figure 2: Lumateperone at 24 hrs of intermediate precision

Limit of detection and limit of Quantification:

Table 5: Data of LOD and LOQ

Test name	values
Limit of detection	0.22 µg/ml
Limit of quantification	0.66 µg/ml

Accuracy:

Table 6: Accuracy data

% Level	Add ppm	% Recovery
ACCURACY_50%	21	100.52
	21	100.89
	21	100.48
ACCURACY_100%	42	101.18
	42	99.63
	42	100.58
ACCURACY_150%	63	99.17
	63	100.00
	63	100.37
	AVG	100.31
	STDEV	0.62
Average % recovery	%RSD	0.62

System Suitability:

Table 7: System suitability data

S No	RT in Min	Area of the peak	USP Plate count	USP Tailing
1	2.579	1428519	2810	1.72
2	2.621	1421736	2798	1.72
3	2.621	1444436	2730	1.72
4	2.629	1429844	2893	1.70
5	2.631	1452117	2857	1.70
6	2.633	1422530	2794	1.72

Robustness:

Table 8: Robustness data

Parameter	%RSD
Flow minus	0.4
Flow plus	0.2
Mobile phase minus	0.3
Mobile phase plus	0.5
Temperature minus	0.4
Temperature plus	0.2

Degradation studies:
Acid Degradation studies:

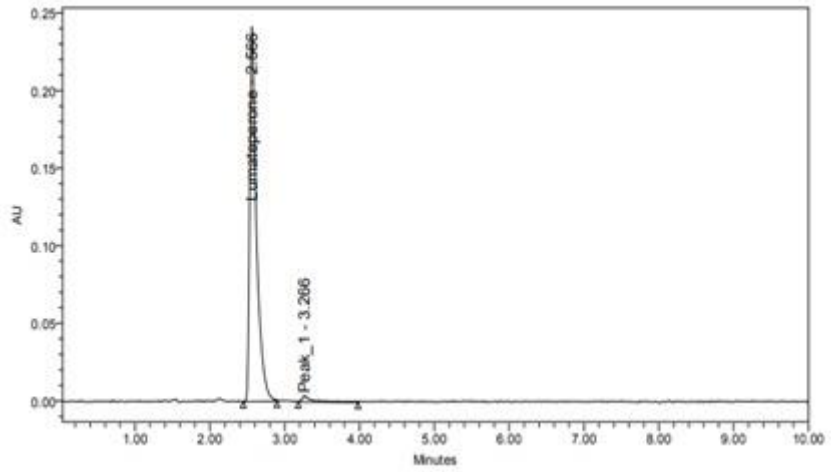

Figure 3: Acid degradation chromatogram

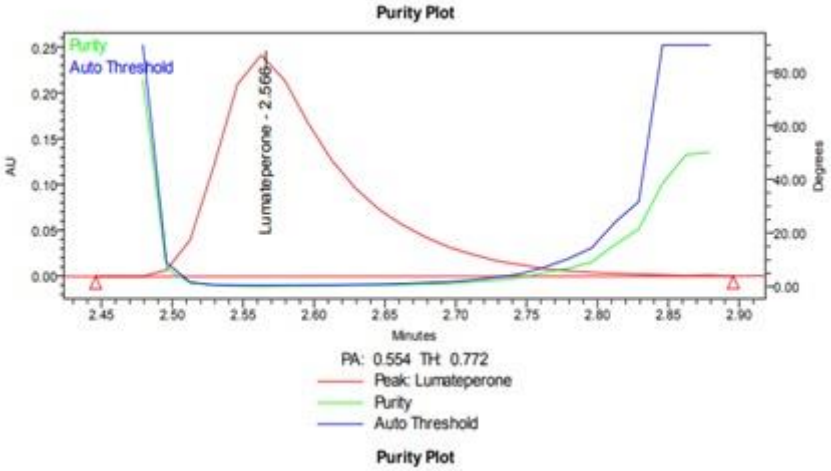

Figure 4: purity plot for Acid degradation study

Alkali degradation studies:

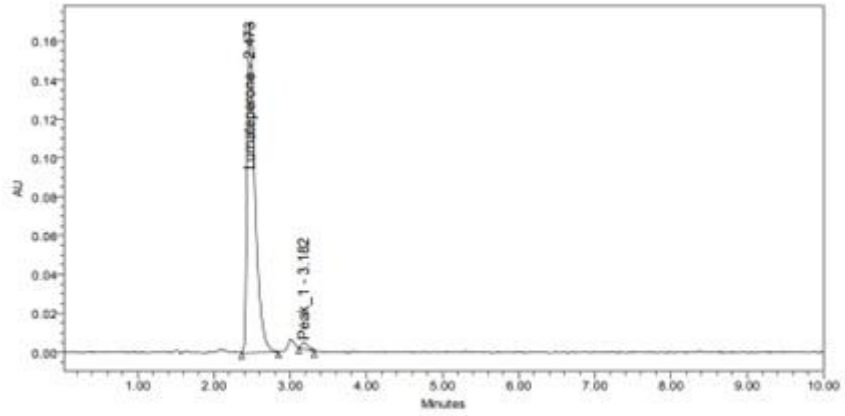

Figure 5: Alkali degradation chromatogram

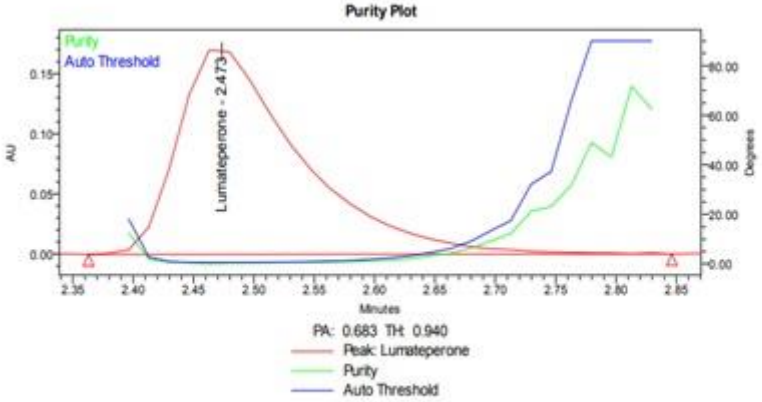

Figure 6: Purity plot for Alkali degradation study

Peroxide degradation studies

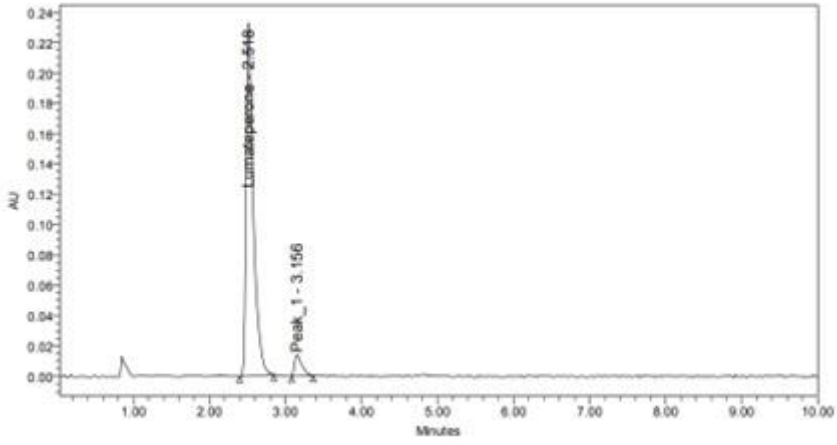

Figure 7: Peroxide degradation chromatogram

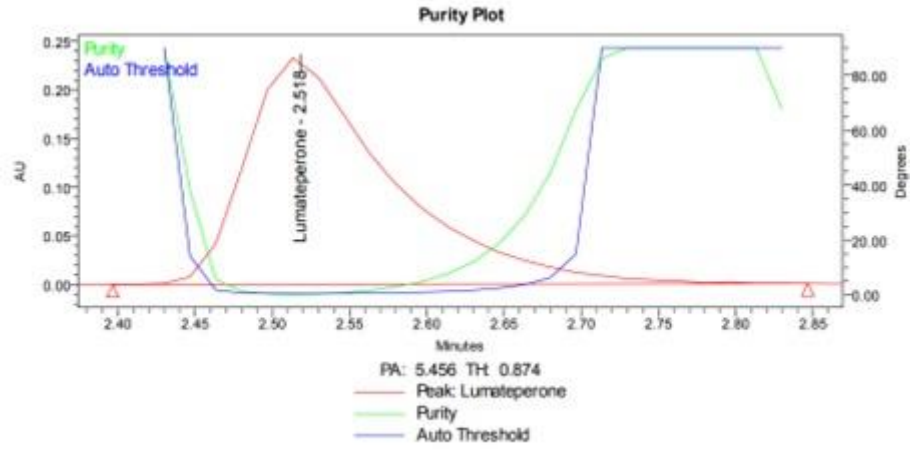

Figure 8: Purity plot for peroxide degradation study

Photolytic degradation study:

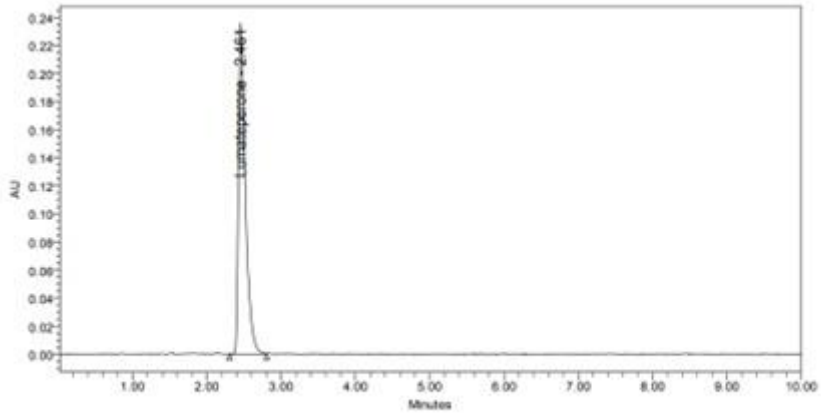

Figure 9: Photolytic degradation study chromatogram

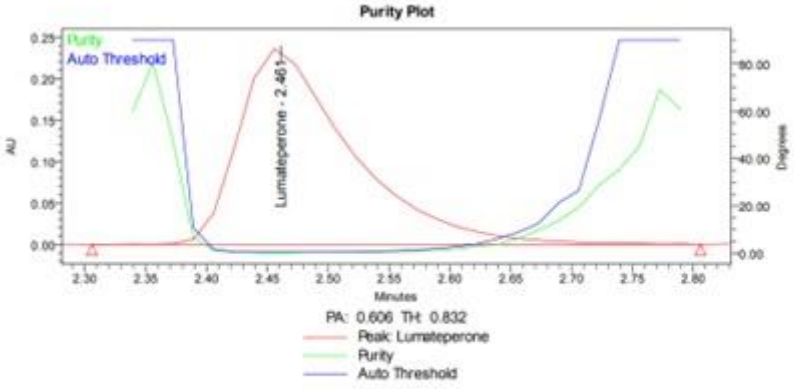

Figure 10: Purity plot for photolytic degradation study

Neutral degradation study:

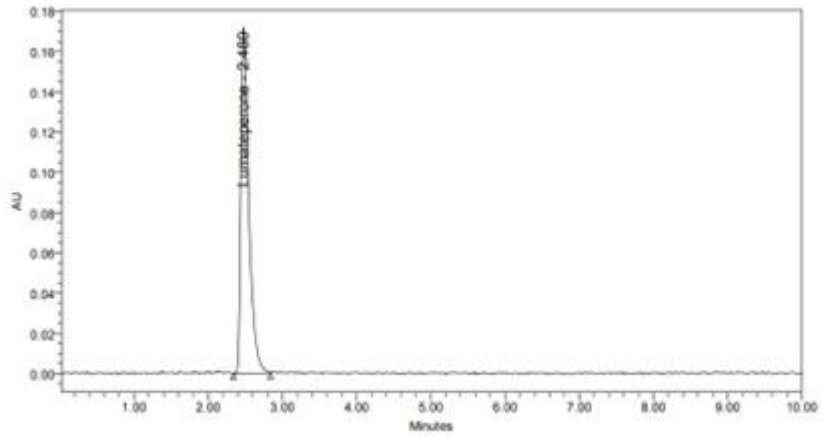

Figure 11: Neutral degradation study chromatogram

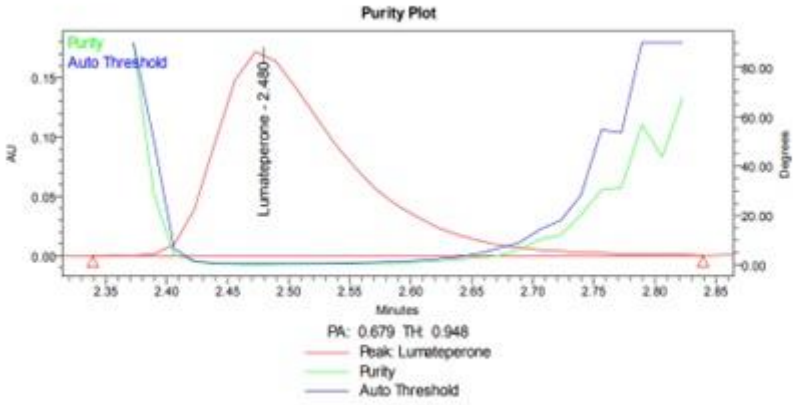

Figure 12: Purity plot for neutral degradation study