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Original Article

Green Metric Based Thin-Layer Chromatography Coupled with Mass Spectrometry to Identify Degradation Products of Lumateperone in Bulk and Formulation

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ABSTRACT

Objectives: The goal of current work was to develop a new, simple, reliable and sensitive High-Performance Thin-Layer Chromatography (HPTLC) method for the estimation of lumateperone. This study also includes the identification of degradation products of lumateperone using different stress conditions and characterization of degradants using mass spectrometry. **Materials and Methods:** The method development was performed using Thin-Layer Chromatography (TLC) plates (pre-coated) with silica gel G 60 F_{254}. The mobile phase comprised ethyl acetate, methanol, toluene and ammonia in a ratio of 4:2:4:0.1 (v/v). Densitometric scanning was carried out at the absorbance mode of 252 nm. Validation was performed according to International Council for Harmonization (ICH Q2R1) guidelines. **Results:** Linearity was achieved in the range of 84-504 ng/band. The calibration curve, R^2=0.9994. The Detection Limit (LOD) and Quantification Limit (LOQ) were identified at 3.22 ng/band and 7.78 ng/band. Recovery ranged from 98% to 102%. Forced degradation results of lumateperone show it is susceptible to degradation in acidic, alkaline, oxidative, thermal, photolytic and neutral stressors. Degradation Products (DPs) of acidic, alkaline and thermal conditions were distinguished by High-Resolution Mass Spectrometry (HR-MS). The greenness of method was confirmed by AGREE tool by following the green chemistry principals. **Conclusion:** The result produced by above method is linear, precise and accurate, making it suitable for routine quantification and stability analysis of lumateperone.

Keywords: HPTLC, HR-MS, Lumateperone, Tablet formulation, Validation.

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INTRODUCTION

Lumateperone is an atypical antipsychotic drug derived from butyrophenone and is used to treat schizophrenia and manic illness (Chavan and Dolas, 2024). chemically, it is 1-(4-fluorophenyl)-4-[(10R, 15*S*). -4-methyl-1, 4, 12-triazatetracyclo [7.6.1.05, 16.010, 15] hexadeca-5, 7, 9(16)-trien-12-yl] butan-1-one (Corponi *et al.*, 2019). Lumateperone is a second-generation antipsychotic drug administered orally that reaches peak plasma concentrations within 1-2 hr (Depietro, 2023). It is a revolutionary investigational drug that affects serotonin, dopamine and glutamate levels in mental condition (Dhami *et al.*,2021). It shows its activity as a serotonin receptor antagonist and exhibits a decreased affinity for dopamine receptors (Edinoff *et al.*,2020; Gamel *et al.*, 2021). Lumateperone has a very high binding affinity (60-fold) for the serotonin receptor compared to the Dopamine (D2) receptor. It also acts on serotonin transporters to reduce serotonin levels, resulting in sleep disorders and schizophrenia (Giulio *et al.*,2023; Howell *et al.*,2015).

High-Performance Thin-Layer Chromatography (HPTLC) is a very effective and advanced analytical approach. It has several benefits, including low operating costs and good sample performance, which have made it a standard analytical technique in the modern period. The main superiority of HPTLC in contrast to High-Performance Liquid Chromatography (HPLC) is that HPTLC processes several samples with the least amount of mobile phase, which lowers processing time and cost per study (ICH Q1A R2). The literature provides a number of analytical techniques used to estimate lumateperone, RP-HPLC (Kamboj *et al.*, 2017; Maini *et al.*, 2021) and stability-indicating investigations (McIntyre *et al.*, 2023). According to the presented data, no literature exists for lumateperone estimation using the HPTLC approach; thus, there is a need to develop a low-cost, sensitive HPTLC method for lumateperone. The current study

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aimed to develop and validate (ICH Q1A R2 guideline., 2003) a new stability-indicating HPTLC method for lumateperone, as well as to characterize the degradation products produced during the stability study using mass spectrometry.

MATERIALS AND METHODS

Reagents and Chemicals

Lumateperone (purity 98.26%) was procured from Sun Pharmaceutical, Mumbai. Lumateperone tablet (42 mg) were formulated in college laboratories. Analytical grade solvents and reagents (Loba Chemie Pvt. Ltd.,) were used.

Instruments

The sample applicator made up of Camag Linomat V, with pressure 3.5 bar, having dimensions of 360 mM×510 mM×410 mM (Width×Length×Height). The stationary phase comprised silica gel (60) F254 plates supplied by Merck, Mumbai, India, with a Camag 100 µL syringe (Hamilton, Switzerland). Development chamber made up of a camag twin glass chamber (10×10 cm and 10×20 cm).

Chromatographic Conditions

The prewashed (methanol) plates were used and dried for 5 min at 100°C in the oven. The mobile phase was developed by trying different mobile phases, comprising ethyl acetate, methanol, toluene, and ammonia in a ratio of 4:2:4:0.1 v/v. Samples were spotted using a Camag Linomat sample applicator and a microliter syringe to create 6-mM-wide bands on precoated silica plates. The plates were dried using an air dryer at 252 nm.

Standard stock solution

The accurately weighed 4.2 mg of Lumateperone was taken into volumetric flask (10 mL) with methanol. Then it was sonicated for 15 min, the further solutions were prepared to get 420 µg/mL concentration.

Optimization of HPTLC method

The HPTLC conditions were optimized with efficient separation of Lumateperone, Mobile phase, comprised of ethyl acetate: methanol: toluene: ammonia (4:2:4:0.1 v/v) since it was determined to have the optimum system suitability characteristics, R_f value was 0.61 at a wavelength of 252 nm.

Formulated Tablets Analysis

The twenty tablets of Lumateperone, having 42 mg of API per tablet, were taken. The average weight of the tablet was determined and then it was crushed into the powder. The tablet weight equivalent to 42 mg of lumateperone was transferred in a 10 mL volumetric flask containing 4 mL of methanol. It underwent sonication for 20 min and was diluted to make the volume 420 µg/mL using methanol. After sonication, the solution was carefully filtered using 0.45 µM Whatman filter paper. The 0.6 µL spots were applied on a TLC plate (pre-coated) with the mobile phase; the TLC plates were developed and analyzed.

Validation of Method

The method was validated using ICH Q2R1 guideline for analysis of the following parameters.

Linearity and Range

For the linearity, a series of Lumateperone solutions from 84 -504 ng/band were prepared from standard and applied on a TLC plate. Following that, TLC plates were activated, dried and analysed using identical chromatographic parameters. The curve was plotted by using Lumateperone concentrations versus peak area.

Precision

The precision study was determined by repeating the sample analysis with different time intervals: intraday precision (same day) and inter-day precision (next day). The % RSD was determined.

Accuracy

The method's accuracy was performed by spiking the dosage form with Lumateperone standard and analyzing the recovery at 3 concentrations: 80%, 100% and 120%. It was investigated three times; the accuracy was expressed as the % recovery.

Detection Limit and Quantification Limit

The detection and quantification limit of proposed method were performed using the formula from the slope and the standard deviation of the intercept. Formula for calculation for LOD and LOQ is given below.

$$LOD=\frac{3.3\times\sigma}{S}\quad LOQ=\frac{10\times\sigma}{S}$$

Where σ=Standard deviation of calibration curve.

S=Slope of the calibration curve.

Robustness

Robustness was assessed by making a few minor, deliberate adjustments to the chromatographic conditions. Saturation time of 5, 10 and 15 min, mobile phase composition and variation in development chamber volume of mobile phase. The % RSD was calculated for all parameters with changes in R_f values and peak area.

Specificity

Specificity was carried out by examining standard lumateperone and lumateperone samples taken from the dosage form; the method's specificity was ascertained by comparing the reflected band's absorbance spectra shown in (Figure 1a and 1b) for

specificity and overlay of Lumateperone standard and formulated Lumateperone tablet. Summary of all validation parameter is provided in Table 1.

Degradation Studies

Lumateperone was exposed to stress degradation in acidic, basic, oxidative, neutral, thermal and photolytic conditions.

Acidic Condition

The 4.2 mg of Lumateperone API was weighed accurately with 3 mL (0.01 N) HCl. The mixture was kept for 20 min at room temperature and then it was neutralized with (0.01 N) NaOH. The volume was made up to the mark with methanol to achieve the concentration of 420 µg/mL.

Basic Condition

The 4.2 mg of Lumateperone was weighed and 3 mL of (0.01 N) NaOH was added. Then it was kept aside for 10 min at room temperature. Methanol was added to the solution to dilute it further to make the concentration of 420 µg/mL.

Oxidative Condition

The 4.2 mg of Lumateperone was weighed, 3 mL of 3% hydrogen peroxide was added. Then it was kept aside for 30 min at room temperature. Methanol was added to the solution to dilute it further to the mark (concentration of 420 µg/mL).

Neutral Condition

The 4.2 mg of Lumateperone was weighed and 3 mL of water was added. Then it was kept aside for 30 min at room temperature. Methanol was added to the solution to dilute it further to the mark (concentration of 420 µg/mL).

Thermal Condition

The 4.2 mg of lumateperone was weighed and lumateperone was heated to 40°C in an oven. Following a 5-min duration, the API was dissolved with methanol and then it was diluted to the desired level (420 µg/mL).

Photolytic degradation

The 4.2 mg of lumateperone was weighed and then it was kept under UV light for a day. Following a 24 hr. Then it was dissolved in methanol and diluted to the desired quantity (420 µg/mL). From the above-prepared sample, 0.6 µL was applied on HPTLC plates to achieve a 252 ng/band concentration (Nakka *et al.*, 2024; Rathod, 2023).

Preparative isolation and characterization of degradation products

Lumateperone (252 ng/band) was spotted on a 0.6 µL TLC plate for the acidic, alkaline and thermal degradation investigation. Plates were prepared and mobile phase was optimized. Study results using mass spectroscopy revealed degradation products. The identification of every divided fragment in the degradation sample is aided by MS examinations.

Tablet Formulation

The marketed preparation of lumateperone was not available in market. So, the tablet was formulated in college laboratory using 42 mg Lumateperone by wet granulation method.

RESULTS

Wavelength Selection

The UV spectra of Lumateperone was recorded in methanol and maximum absorbance was seen at 252 nm.

Chromatographic system

To analyze the Lumateperone (bulk dosage form and degradation samples) the optimized mobile phase was used i.e. ethyl acetate: methanol: toluene: ammonia (4:2:4:0.1v/v). The plate was developed in a mobile phase (optimized). Good peak shape and resolution between the sample and degradation products were obtained, with an acceptable R_f =0.61.

Table 1: Summary of validation parameters.

Sl. No.	Specifications	Validation Results
1.	Linearity (*n*=6)	84 to 504 ng/band
2.	Regression Equation	y=4.1883x+94.253
3.	Correlation coefficient (R^2)	0.9994
4.	Accuracy (*n*=3)	% Recovery
	80%	101.3
	100%	101.8
	120%	102.0
5.	Precision (ng/band) Intra-day (*n*=3)	Mean±%RSD
	168	897.9±0.44
	252	1248.9±0.78
	336	1557.2±0.56
	Precision (ng/band) Inter-day (*n*=3)	
	168	897.9±0.42
	252	1245.8±0.72
	336	1541.3±0.47
6.	LOD (ng/band)	3.22 ng/band
7.	LOQ (ng/band)	9.78 ng/band
8.	Robustness (*n*=3)	% RSD
	Mobile phase	0.51±0.32
	Saturation time	0.45±0.35
	Development chamber volume of mobile phase	0.63±0.38

Validation

Summary of developed validation parameters

The presented HPTLC technique is simple, accurate and robust for analysis of Lumateperone and it was shown based on results of validation parameters; it proved the reliability of the method. Summary of validation is provided in Table 1.

Analysis of formulated tablet

The Lumateperone drug concentration was determined between range 98.9 to 100.8% in the formulation trials as shown in Table 2. This method can be used for routine drug estimation.

Stress degradation studies

The stress degradation analyses were determined by acid, alkali, oxidative, thermal, photolytic and neutral degradation of the

Table 2: Formulated Tablet analysis.

Sl. No.	Quantity of API taken (ng/band)	Peak area	Quantity of API recovered (ng/band)	% Recovery
1	252	1240.2	249.4	98.9
2	252	1263.5	254.1	100.8
3	252	1239.1	249.1	98.8
4	252	1244.8	252.3	99.3
5	252	1258.6	254.2	100.4
6	252	1264.8	251.7	100.8
Mean		1251.75	251.74	99.89
Standard Deviation (SD)				11.6
% Relative Standard Deviation (% RSD)				0.93

Table 3: Summary of degradation products of Lumateperone.

Sl. No.	Type of degradation	Stressors condition	R_f value of degradants	% Degradation	% Recovered
1.	Acidic degradation	0.01 N HCl, for 20 min at room temperature.	0.8	19.95	80.05
2.	Alkaline degradation	0.01N NaOH for 15 min at room temperature.	0.58	26.28	73.72
3.	Oxidative degradation	3% H_2O_2, for 30 min at room temperature.	0.79	13.8	86.2
4.	Thermal degradation	40°C in oven for 15 min	0.78	21.9	78.1
5.	Photolytic degradation	Exposure to UV light for 24 hr.	0.81	11.9	88.9
6.	Neutral degradation	H_2O for 30 min, at room temperature.	0.15	10.6	89.4

Table 4: Summary of degradation Products of Lumateperone using MS.

Sl. No.	Name of Compound	Molecular weight in g/mol	Molecular formula	Name of compound (IUPAC)
1	Lumateperone	393	$C_{24}H_{28}FN_3O$	1-(4-Fluorophenyl)-4-[(10R,15S)-4-methyl-1,4,12-triazatetracyclo [7.6.1.05,16.010,15] hexadeca-5,7,9(16)-trien-12-yl] butan-1-one.
2	DP 1	214	$C_{13}H_{15}N_3$	Imine Derivative
3	DP 2	214	$C_{13}H_{16}N^+_3$	Iminium Derivative
4	DP3	224	$C_{13}H_{19}FN^+O$	Ammonium Derivative 4-(N-ethyl-N-methylammonium)-1-(4-fluorophenyl) butan-1-one.
5	DP 4	257	$C_{16}H_{15}N^+_3$	Iminium Derivative

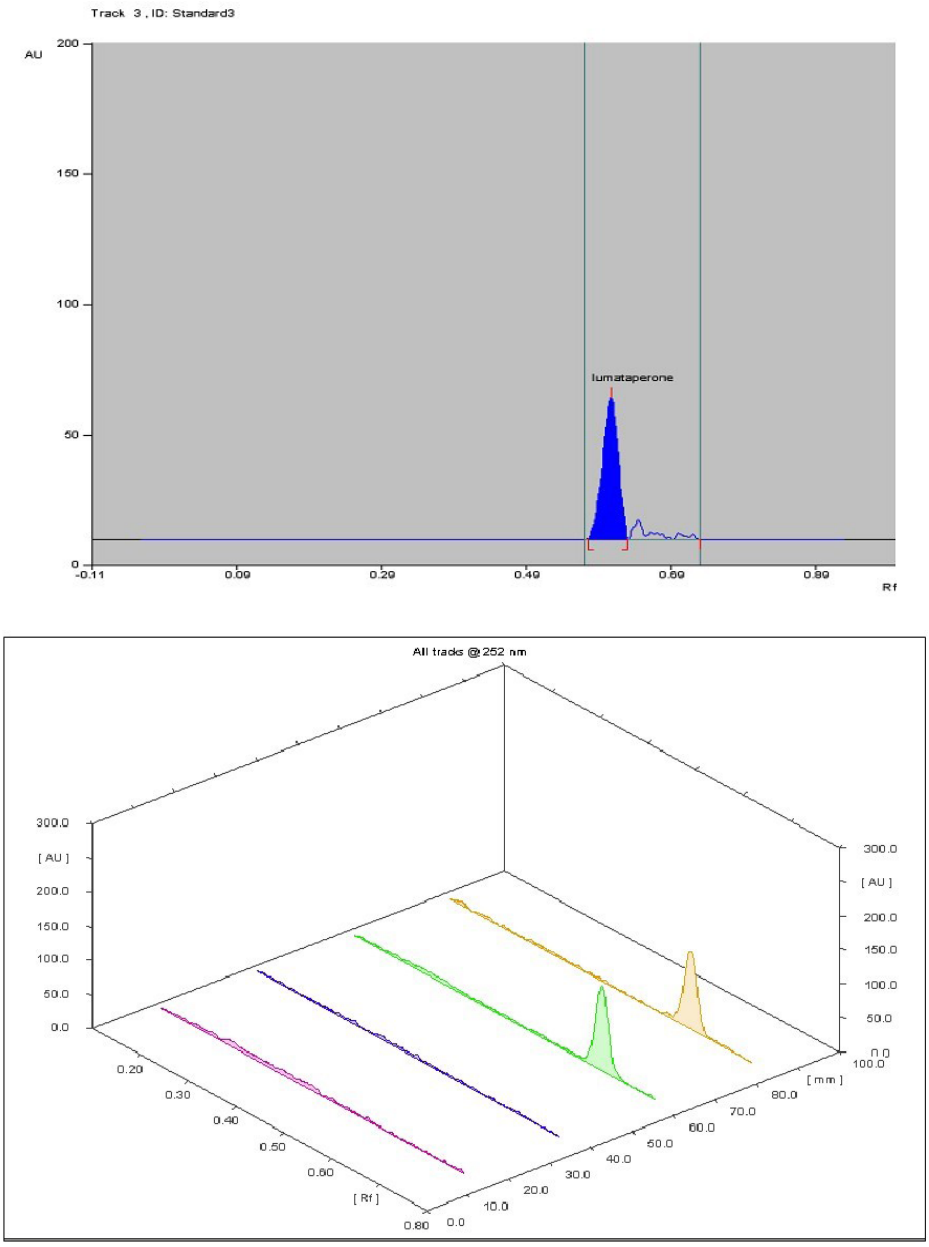

Figure 1: a) Optimized densitogram of lumateperone, b) 3D Spectra for Specificity.

Lumateperone standard. well-separated, pure Lumateperone bands with a specific R_f value were discovered. Table 3 presents the results and Figure 2 displays a densitogram of deterioration at 252 nm. It was shown that the drug degraded in almost every kind of stress, including alkali, hydrolytic, oxidative and acidic stressors. The significant percentage of degradation was observed in photolytic stress.

Characterization of Degradation Products

The studies were performed in various stress conditions and degradation products thus obtained were characterized by mass spectrometry. Lumateperone has an m/z value of 393.5.

The acidic-degraded solution of the drug was characterized using mass spectrometry, shown in Figure 3a, revealing two degradants. Lumateperone, with an m/z value of 393.5, undergoes cleavage at Fluorophenyl butan-1-one ($C_{11}H_{13}FO$) and ($C_{11}H_{12}FO$) to yield an imine derivative as degradation product 1 (DP1) with an m/z value of 213 (M+H=214.3) and an iminium Derivative (DP2) with an m/z value of 214 (M+=214.8). The degradation pathway is shown in Figure 3b.

The alkali-degraded solution of the drug shown in Figure 4a reveals one degradation product of Lumateperone, with an m/z value of 393.5, which undergoes removal of the amino piperidine ring ($C_{11}H_9N_2$) to yield Degradant Product 3 (DP3),

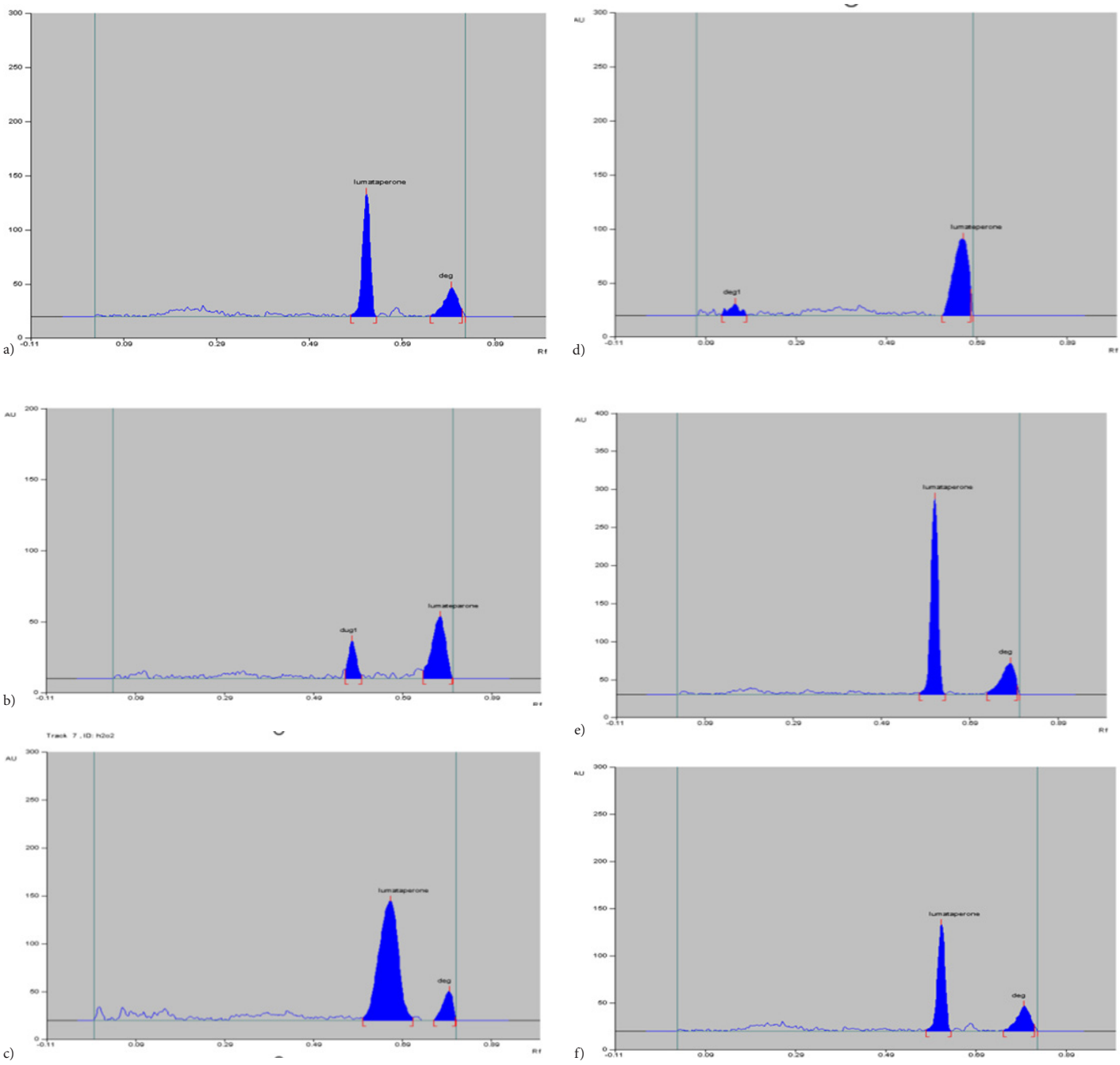

Figure 2: Densitogram of lumateperone degradation conditions (a) Acid, (b) Base, (c) Hydrogen peroxide, (d) Neutral, (e) Photolyticand and (f) Neutral.

the ammonium derivative, which has an m/z value of 224 (M+=224.6). The degradation pathway is shown in Figure 4b.

The thermally degraded solution of the drug shown in the spectra of Figure 5a reveals the Degradation Product (DP4) lumateperone with an m/z value of 393 that undergoes removal of 4-fluorophenyl and carbonyl group (C_8H_6FO) to yield a stable fragment iminium derivative, i.e., DP4, with an m/z value of 256.18 (M+H=257.6). Degradation pathway illustrated in Figure 5b. The summary of Lumateperone and its degradation products are shown in Table 4

The greenness of the above method was assessed using the AGREE program, which offers reliable and precise findings for method greenness. Following the input of all 12 green chemistry principles into software on a 0 to 1 scale, which is indicated by a red, yellow, or green color, the graph is generated automatically by the software. The entire result is displayed centrally, i.e., 0.72, with a value closer to one and a darker green signifying how

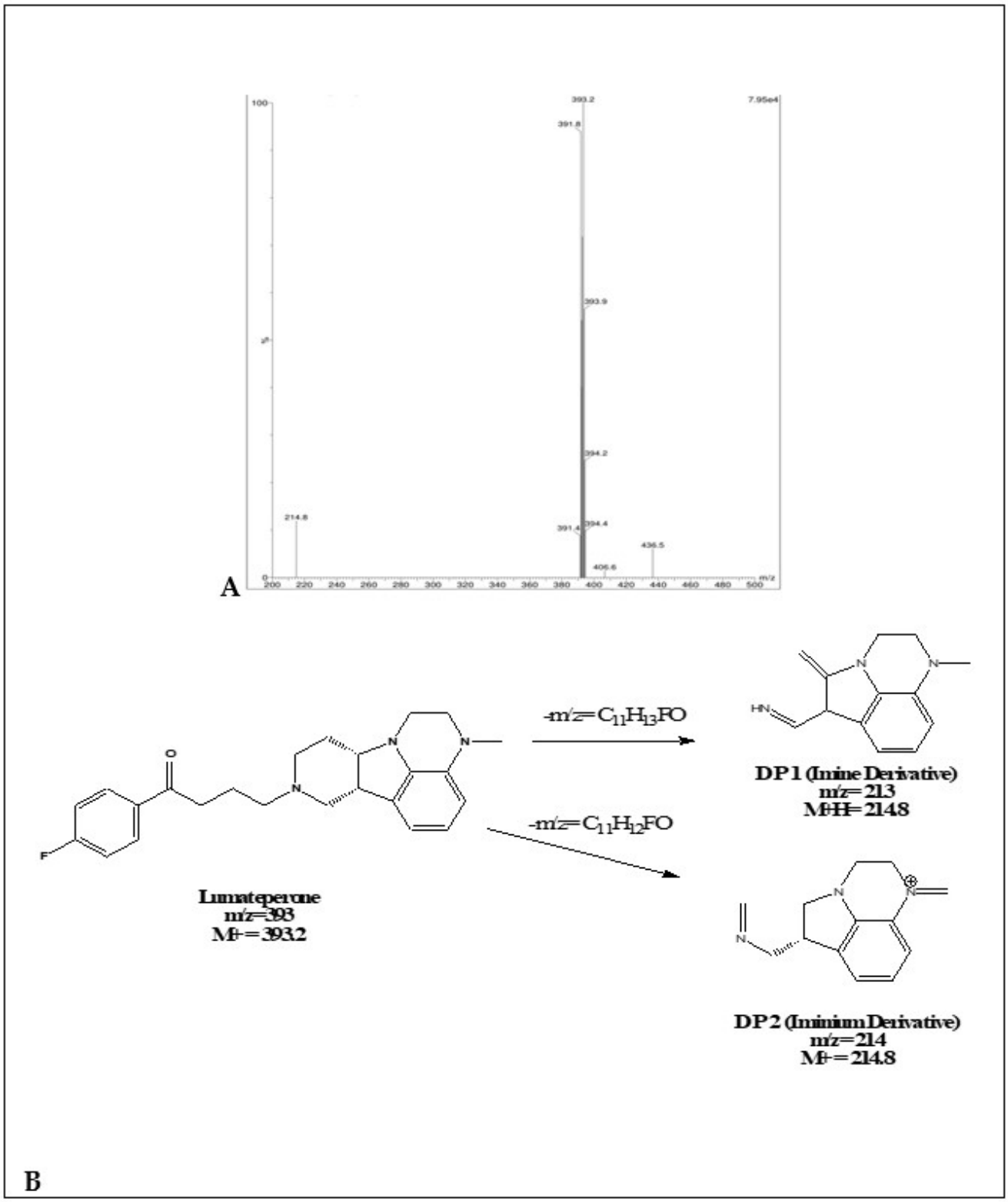

Figure 3: a) Mass spectra of acidic degradation for Lumateperone. b) Degradation of Lumateperone in acidic medium

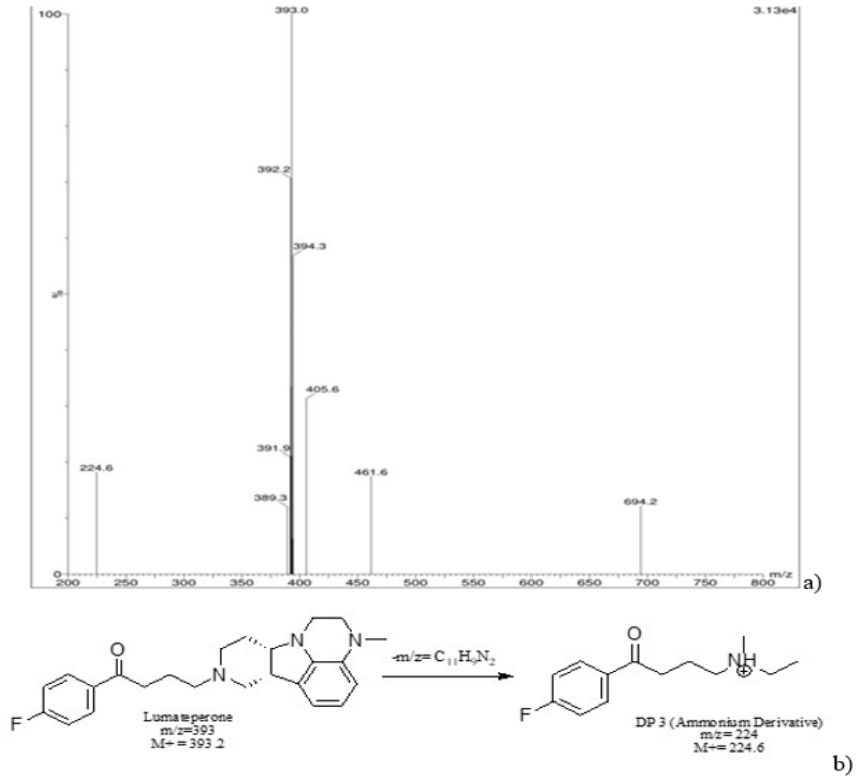

Figure 4: a) Mass spectra of basic degradation for Lumateperone. b) Degradation of Lumateperone in alkaline medium.

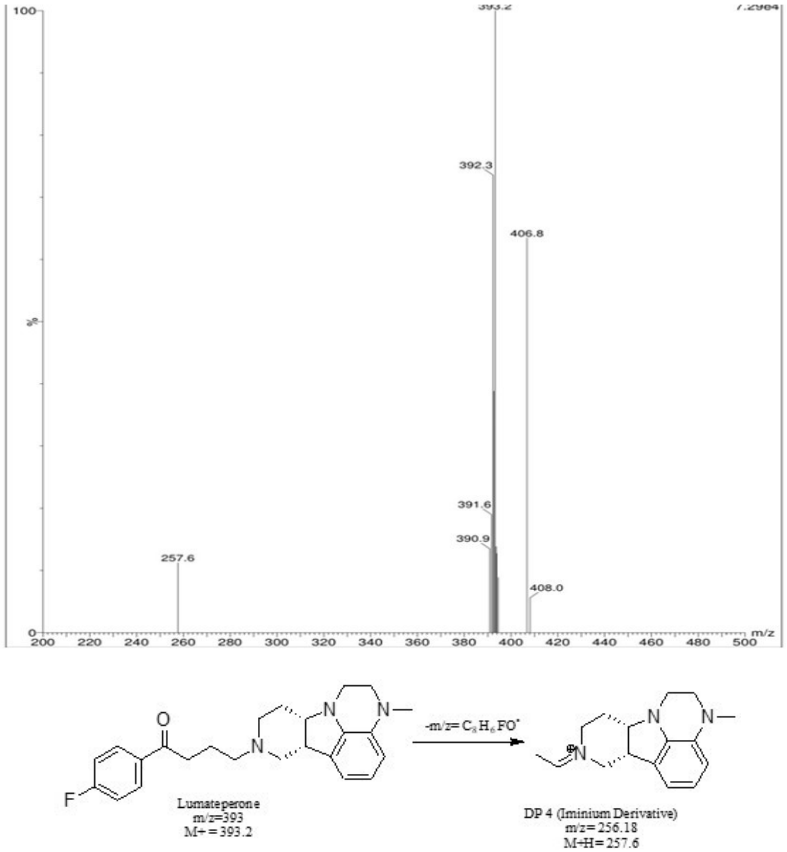

Figure 5: a) Mass spectra of thermal degradation for Lumateperone. b) Degradation of Lumateperone in thermal medium.

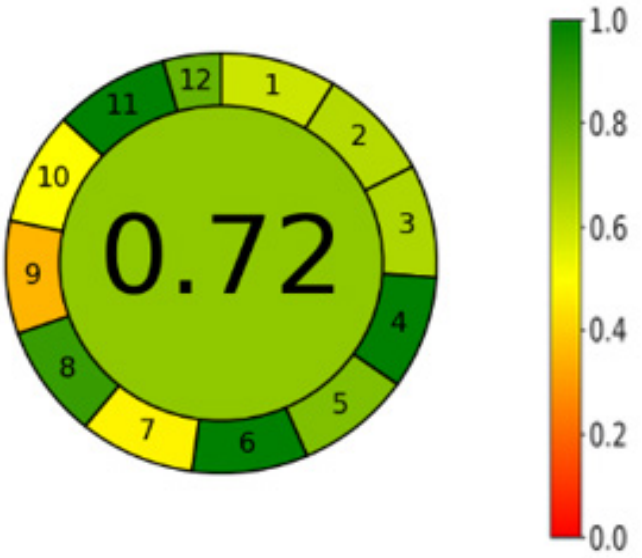

Figure 6: Greenness of method.

much greener and more eco-friendly the process is (Figure 6). The estimated score of 0.72 indicates that the analyzed method is more ecologically friendly (Shivaji, 2023).

DISCUSSION

As there are no reported HPTLC methods available for Lumateperone estimation, hence the new HPTLC method development and validation of Lumateperone was performed according to ICH Q2R1 guidelines. The detailed investigation of degradation under various stress conditions was performed and degradants were characterized by mass spectrometry.

The results of proposed methods show the mobile phase was optimized, ethyl acetate: methanol: toluene: ammonia (4:2:4:0.1 v/v) at the 252 nm absorbance. RF value was obtained at 0.61. Linearity was performed between a range of 84-504 ng/band, with R^2=0.9994; recovery was achieved between 98.8% and 100.8%.

The stability results show the drug is susceptible to acidic, basic, alkaline, photolytic, thermal and neutral conditions (Table 3). The degradation product obtained DP1 as an imine derivative at 214 *m/z* value, DP2 as 4-(*N*-ethyl-*N*-methylammonium)-1-(4-fluorophenyl) butan-1-one at 224 *m/z* value and DP4 as an iminium derivative at 257 *m/z* value were identified and distinguished by HR-MS with their proposed drug degradation pathways.

CONCLUSION

A new, robust, sensitive and precise HPTLC method of lumateperone was optimized and validated using ICH Q2R1 guidelines. Also, the mass spectra of lumateperone with its degradation products under different stress conditions were analyzed with the degradation pathways using mass spectrometry. The above methods found the potential green method for the quality analysis for lumateperone tablet formulation.

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ABBREVIATIONS

HPTLC: High Performance Thin-Layer Chromatography; **MS:** Mass Spectrometry; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **SD:** Relative Standard Deviation; **ICH:** International Council for Harmonization; **DP:** Degradation Product.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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