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Development and validation of a stability-indicating RP-UPLC method for the estimation of loxapine in bulk drug and pharmaceutical dosage form

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Abstract
A rapid and reliable ultra-performance liquid chromatographic (UPLC) method was developed and validated for the quantitative estimation of Loxapine in bulk drug and pharmaceutical dosage form. The method was developed using a Waters ACQUITY UPLC H-Class system equipped with a photodiode array detector. Chromatographic separation was achieved on an ACQUITY BEH C18 column (2.1 × 50 mm, 1.7 µm) using acetonitrile and phosphate buffer (pH 3.0) containing 0.1% triethylamine in a 65:35 (v/v) ratio as the mobile phase. The flow rate was 0.3 mL/min, detection was performed at 254 nm, injection volume was 2 µL, and the total run time was 8 min. Loxapine showed a retention time of 3.898 min with 4596 theoretical plates and a tailing factor of 0.87. The method was validated for system suitability, specificity, sensitivity, linearity, precision, accuracy, ruggedness, robustness, and forced degradation. Linearity was established over 2.5–15 µg/mL with r^2 = 0.99978. The LOD and LOQ were 0.257 and 0.777 µg/mL, respectively. Accuracy studies showed recoveries of 98.50–99.06% with %RSD values below 2%. System precision and method precision showed %RSD values of 0.71% and 0.70%, respectively. The method demonstrated acceptable ruggedness and robustness. Forced degradation showed maximum degradation under acid stress (4.45%), followed by alkali (3.55%) and oxidative stress (3.20%), while neutral hydrolysis, thermal, and photolytic conditions produced comparatively lower degradation. The assay was 98.92% for the bulk drug and 99.99% of label claim for the marketed capsule formulation. The developed method was therefore found to be suitable for routine quantitative analysis of Loxapine in bulk drug and pharmaceutical dosage form.

Keywords: Loxapine, UPLC, method development, method validation and forced degradation

Introduction

Loxapine is an antipsychotic drug used in the management of schizophrenia and acute agitation associated with schizophrenia or bipolar disorder. The supplied drug profile identifies Loxapine as 8-chloro-6-(4-methylpiperazin-1-yl) benzo [b] [1, 4] benzoxazepine, with molecular formula C18H18ClN3O and molecular mass 327.81 g/mol. It is described as a pale-yellow crystalline solid or light-yellow powder and is sparingly soluble in water but soluble in organic solvents such as DMSO. [1–4]

The supplied experimental study was undertaken to develop and validate a UPLC method for estimation of Loxapine in bulk drug and a marketed pharmaceutical formulation. Method development involved variation of chromatographic conditions to obtain a symmetric peak, adequate chromatographic performance, and a short analysis time. The final method was subsequently validated for the principal analytical characteristics and evaluated under forced-degradation conditions. [5, 6]

Mateials and methods

Instrumentation

Chromatographic analysis was performed using a Waters ACQUITY UPLC H-Class system equipped with a PDA detector, binary solvent manager, and auto-injector. Empower 3 chromatography data software was used for data acquisition and processing. Other laboratory equipment included a Sartorius BSA224S-CW analytical balance, Eutech pH 700 pH meter, UCA 701 ultrasonic bath, borosilicate vacuum filtration kit, 0.22 µm Merck Millipore membrane filters, hot air oven, and photostability cabinet.

Chemicals and Reagents

Loxapine succinate working standard (99% purity) was obtained as a gift sample from Jiyan Chemicals and Pharmaceuticals, Gujarat. Loxapine succinate capsules with a 10 mg label claim were purchased from a local pharmacy. HPLC-grade acetonitrile, methanol, and water, potassium dihydrogen phosphate, orthophosphoric acid, and triethylamine were procured from Merck Chemicals Private Limited, Mumbai.

Preparation of 0.01 M Phosphate Buffer (pH 3.0)

Accurately weighed potassium dihydrogen phosphate (1.36 g) was dissolved in approximately 800 mL of HPLC-grade water in a 1000 mL volumetric flask. The solution was sonicated/stirred until dissolution was complete. The pH was adjusted to 3.0 ± 0.05 using orthophosphoric acid, the volume was made up to 1000 mL with HPLC water, and the buffer was filtered through a 0.22 µm membrane filter.

Preparation of Mobile Phase
Acetonitrile and phosphate buffer (pH 3.0) containing 0.1% v/v triethylamine were mixed in a 65:35 (v/v) ratio. Triethylamine was included to suppress peak tailing associated with the basic piperazine nitrogen of Loxapine. The mobile phase was sonicated for 15 min and filtered through a 0.22 µm membrane filter before use.

Preparation of Standard Solution
Loxapine succinate (10 mg) was accurately weighed and transferred into a 10 mL volumetric flask. Approximately 7 mL of acetonitrile was added and the material was completely dissolved using an ultrasonic bath. The volume was made up to the mark with the same diluent and the solution was filtered through a 0.22 µm membrane filter to obtain a 1000 µg/mL stock solution. Working standards were prepared by serial dilution using the mobile phase as diluent.

Preparation of Formulation Sample
Twenty marketed Loxapine succinate capsules (10 mg label claim) were weighed and the average fill weight was determined. Powder equivalent to 10 mg of Loxapine was transferred into a 10 mL volumetric flask. Approximately 7 mL of acetonitrile was added and the mixture was sonicated for 15 min. After cooling to room temperature, the volume was made up to the mark with the same diluent. The solution was filtered through a 0.22 µm membrane filter after discarding the first few millilitres of filtrate. Appropriate aliquots were diluted with mobile phase to obtain the required working sample concentration.

Method development
A standard Loxapine solution was scanned from 200 to 400 nm using the PDA detector, and 254 nm was selected as the detection wavelength based on maximum absorbance. Several chromatographic trials were conducted by varying the mobile phase composition, column, and flow rate. The optimized condition produced a symmetric peak with acceptable chromatographic efficiency.

Table 1: Optimized Chromatographic Conditions

Parameter	Optimized condition
Column	ACQUITY BEH C18 (2.1 × 50 mm, 1.7 µm)
Mobile phase	Acetonitrile: phosphate buffer pH 3.0 with 0.1% TEA (65:35 v/v)
Flow rate	0.3 mL/min
Detection wavelength	254 nm
Injection volume	2 µL
Temperature	Ambient
Run time	8 min

Method validation
The developed UPLC method was validated for system suitability, specificity, LOD, LOQ, linearity, precision, accuracy, ruggedness, and robustness. System suitability was evaluated from six replicate standard injections. The specified acceptance criteria included theoretical plates greater than 2000, tailing factor below 2, resolution greater than 2 where applicable, and peak-area %RSD below 2%.
Specificity was evaluated using blank and placebo solutions to confirm the absence of interfering peaks at the Loxapine retention time. LOD and LOQ were calculated from the residual standard deviation and slope of the calibration curve. Linearity was assessed over 2.5–15 µg/mL. Precision included system precision and method precision. Accuracy was assessed by standard addition at 50%, 100%, and 150% levels. Ruggedness was assessed using a second analyst, while robustness was studied by deliberate changes in mobile phase composition and flow rate.

Forced degradation studies
Loxapine was subjected to acid, alkali, oxidative, neutral hydrolysis, thermal, and photolytic stress conditions. Acid hydrolysis was performed using 0.1 N hydrochloric acid at 60 °C for 12 hrs, alkali hydrolysis used 0.1 N sodium hydroxide at 60 °C for 12 hrs, oxidative degradation used 3% v/v hydrogen peroxide for 12 hrs, neutral hydrolysis involved reflux with HPLC-grade water at 60 °C for 12 hrs thermal degradation involved exposure of solid drug to 105 °C for 8 hrs and photolytic degradation involved UV light exposure in a photostability chamber for 8 hrs. Stressed and unstressed samples were analysed under the optimized conditions.

Assay
The bulk drug and marketed capsule formulation were assayed using the validated UPLC method by comparison of sample and reference-standard peak responses under the optimized chromatographic conditions.

Results and discussion
Method Development and Optimization
Five chromatographic trials were performed. Trial 1 showed baseline noise, Trial 2 showed baseline noise and tailing, Trial 3 showed tailing, and Trial 4 showed a merged/unknown peak. Trial 5, using acetonitrile: phosphate buffer pH 3.0 with 0.1% triethylamine (65:35 v/v), produced a symmetric peak and was selected as the optimized condition.

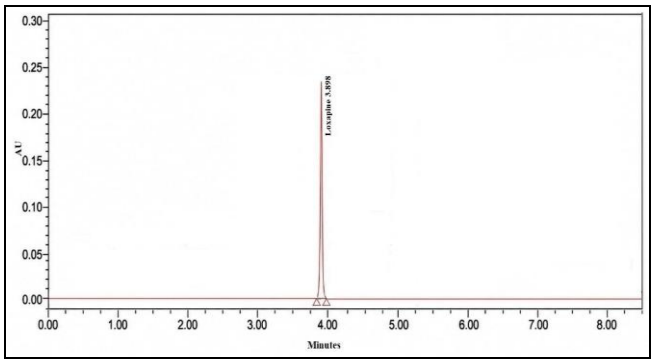

Fig 1: Optimized Chromatogram of Loxapine

Table 2: Results for Optimized Chromatogram

Drug	Retention Time (Min)	Theoretical plates	Tailing factor
Loxapine	3.898	4596	0.87

Six replicate injections gave mean retention time and peak area %RSD values of 0.05% and 0.68%, respectively, both below the 2% criterion, confirming system suitability.

Specificity
Blank and placebo chromatograms showed no interfering peak at the retention time of Loxapine, demonstrating specificity of the developed method.

System Suitability

Table 3: Results for System suitability

Injection	Retention Time(Min)	Peak area
1	3.897	109854
2	3.902	111364
3	3.898	111086
4	3.901	110967
5	3.896	109576
6	3.899	109984
Mean	3.898833	110471.8
SD	0.002317	753.7332
%RSD	0.05	0.68

LOD and LOQ

Table 4: Results for LOD & LOQ

Parameter	Value
Residual SD (σ)	767.16
Slope (S)	9869.55
LOD	0.257 µg/mL
LOQ	0.777 µg/mL

The calculated LOD and LOQ were 0.257 and 0.777 µg/mL, respectively, demonstrating the sensitivity of the method.

Linearity

Table 5: Results for Linearity

Level	Concentration (µg/mL)	Peak area
1	2.5	35976
2	5	61857
3	7.5	86798
4	10	109898
5	12.5	135854
6	15	159675

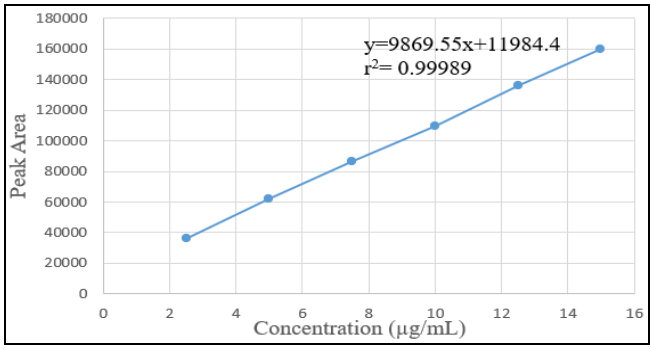

Fig 2: Linearity graph for Loxapine

The calibration curve was linear over 2.5–15 µg/mL. The regression equation was y = 9869.55x + 11984.40, with r = 0.99989 and r^2 = 0.99978.

Precision

System precision produced a peak-area %RSD of 0.71%, while method precision produced 0.70%. Both values were below 2%, demonstrating good precision and reproducibility.

Table 6: Results for System Precision & Method Precision

Parameter	Mean retention Time (min)	Mean peak Area	%RSD (Area)
System precision	3.900	110896.7	0.71
Method precision	3.900	110868.5	0.70

Accuracy

Table 7: Accuracy results for Loxapine

Spiking level	Mean % recovery	%RSD
50%	98.83	0.17
100%	98.66	0.17
150%	98.69	0.10

Recovery ranged from 98.50% to 99.06% across the individual determinations at 50%, 100%, and 150% levels, with %RSD values of 0.17%, 0.17%, and 0.10%, respectively. The results were within the stated acceptance range of 98–102% recovery and below 2% RSD.

Ruggedness

Analysis by a second analyst produced a peak-area %RSD of 0.45%, demonstrating acceptable ruggedness with respect to analyst-to-analyst variation.

Robustness

Small deliberate variations in mobile phase ratio and flow rate resulted in peak-area changes of 0.15–0.31%, with an overall reported %RSD of 0.91%, demonstrating robustness of the method.

Forced Degradation

Table 8: Results for Forced Degradation

Stress condition	Peak area	% Degradation	% Assay
Control	109769	0	100
Acid	104874	4.45	95.55
Alkali	105867	3.55	96.45
Oxidative	106249	3.20	96.80
Hydrolysis	108929	0.76	99.24
Thermal	109103	0.60	99.40
Photolytic	109487	0.25	99.75

Loxapine showed the greatest degradation under acid stress (4.45%), followed by alkali stress (3.55%) and oxidative stress (3.20%). Lower degradation was observed under neutral hydrolysis (0.76%), thermal (0.60%), and photolytic (0.25%) conditions. The Loxapine peak remained resolved from degradation products observed under the stress conditions, supporting the stability-indicating capability of the developed method.

Assay of Bulk Drug

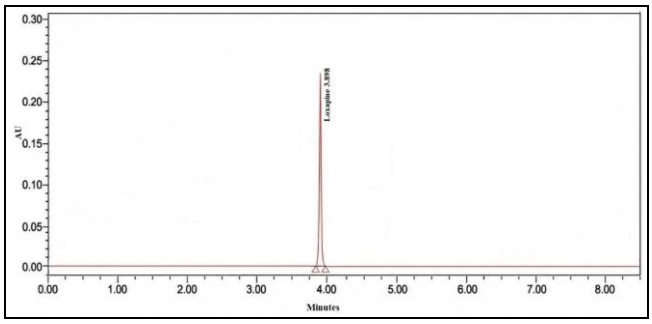

Fig 3: Chromatogram of Assay-API

Table 9: Results for Assay – API

Drug	Peak area	% Assay
Loxapine	108722	98.92

Table 10: Results for Assay – Formulation

Drug	Brand	Label claim	Peak area	Amount found	% Assay
Loxapine	Loxapax	10 mg	109896	9.99 mg	99.99

Assay of Pharmaceutical Formulation

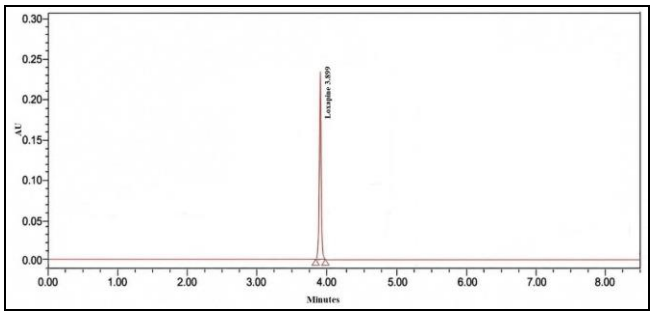

Fig 4: Chromatogram of Assay-Formulation

Conclusion

A simple, rapid, precise, accurate, sensitive, robust, and rugged UPLC method was developed and validated for the estimation of Loxapine in bulk drug and pharmaceutical dosage form. The optimized method provided a retention time of 3.898 min and satisfactory chromatographic efficiency within an 8 min run. The method showed excellent linearity over 2.5–15 µg/mL, low LOD and LOQ, acceptable precision and accuracy, and satisfactory ruggedness and robustness. Forced-degradation studies demonstrated degradation under acid, alkali, and oxidative stress, with comparatively low degradation under neutral hydrolysis, thermal, and photolytic conditions. The assay results of 98.92% for bulk drug and 99.99% for the marketed capsule formulation further support the suitability of the method for routine quantitative analysis of Loxapine

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