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DEVELOPMENT OF UV SPECTROPHOTOMETRIC AND HPTLC TECHNIQUE FOR ESTIMATION OF LOXAPINE IN FORMULATION

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ABSTRACT

UV spectrophotometric and chromatographic methods which are simple, accurate and precise have been developed and validated for the determination of Loxapine in its pharmaceutical formulation. The UV spectroscopic analysis was performed using water as solvent and detection was done at 297 nm. The HPTLC method was performed using Pre-coated silica gel G60 F_{254} plate using the mobile phase chloroform and methanol in the ratio of 9.5:0.5 v/v/v followed by analysis at 297 nm. Linearity was found over the concentration range of 10- 80 µg/ml for UV method and 2 – 12 ng/spot for HPTLC method with correlation coefficient 0.9991 and 0.9933 respectively. Repeatability, Intraday and Interday studies shows % RSD less than 2, which indicates the precision of the developed method. The developed methods are more sensitive and cost effective. As no methods were developed for Loxapine by UV and HPTLC techniques they can be routinely used for the determination Loxapine in formulations.

Keywords: *Loxapine, UV spectroscopy, HPTLC, Method development, Validation.*

1. INTRODUCTION

Loxapine, a dibenzoxazepine compound, represents a subclass of tricyclic antipsychotic agents, mainly used for the management of the manifestations of psychotic disorders such as schizophrenia. It acts as dopamine antagonist, and also serotonin 5-HT2 blocker. Chemically, it is 2-Chloro-11-(4-methyl-1-piperazinyl) dibenz[b,f][1,4] oxazepine and the chemical formula is $C_{18}H_{18}ClN_3O$ [1].

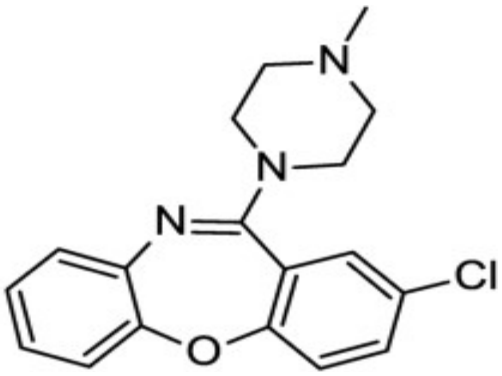

Fig. 1: Chemical structure of Loxapine

The main objective of this study was to develop simple and cost effective analytical method viz UV spectroscopy and high performance thin layer chromatography for the determination of Loxapine in capsule dosage form. The developed methods were validated according to ICH guidelines. The literature survey revealed there are few HPLC analytical methods reported for the determination of Loxapine [1-7]. As there were no reported methods for UV spectroscopy and HPTLC methods for Loxapine, the current study was undertaken and the methods were developed.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The sample of Loxapine was received from Rajesh chemicals limited, Mumbai, India with certificate of analysis. Methanol, chloroform and other solvents were supplied by S.D. Fine chemicals Ltd India and Qualigens fine chemicals Ltd., Mumbai, India. Loxapine capsule dosage forms were purchased from the local market.

2.2 Instrumentation and analytical conditions

According to the experimental conditions of the study, UV spectroscopic analysis was performed on Jasco V – 630 UV/Vis Spectrophotometer and a pair of 1cm quartz cuvette was used to measure the absorbance of the solutions. HPTLC method was developed and validated using CAMAG TLC scanner, Linomat V sample applicator connected to a nitrogen cylinder, a twin trough chamber with WINCATS software. Pre-coated silica gel G60 $F_{254\ plate}$ was used as stationary phase. The UV spectroscopic analysis was performed using water as solvent and detection was done at 297 nm. The mobile phase selected for HPTLC method comprises chloroform and methanol in the ratio of 9.5: 0.5 v/v/v. The optimized chamber saturation time was 15 minutes. The wavelength at which the spots were analyzed was 297 nm.

2.3 Preparation of stock solutions and working standard solutions

i) UV method

Loxapine (10 mg) was accurately weighed and dissolved in water and the final volume was adjusted to 10ml with water to prepare 1000 µg/ml stock solution of the drug. The working standard solutions of concentration ranging from 10 µg/ml to 80 µg/ml were prepared from the stock solution and scanned in the UV region 200 to 400 nm and the absorbance was measured at 297 nm.

ii) HPTLC method

Loxapine (10 mg) was accurately weighed and dissolved in methanol and the final volume was adjusted to 10 ml with methanol to prepare 1000 µg/ml stock solution of the drug. A 100 µg/ml of solution was prepared from the stock solution. From this stock solution 2 µl to 12 µl were spotted on HPTLC plate.

2.4 Analytical method validation

The developed methods were validated as per ICH Q2R1 guidelines [8]. The Parameters validated were linearity, accuracy, precision and repeatability.

2.4.1 Linearity

Six different concentrations of the drug were prepared and checked for linearity in UV spectroscopic method. Loxapine showed good linearity in the concentration range of 10-80 µg/ml and the calibration curve was plotted between absorbance and different concentration of the drug. Calibration graph for HPTLC was obtained by plotting concentration vs peak area of drug ranging from 2 µg - 12 µg/ spot.

2.4.2 Precision

Precision of the method was carried out by intra and inter day precision studies. Intraday precision was found by analyzing three concentrations of standard Loxapine in the linearity range for three times on the same day. Inter- day precision was carried out on three different days using three replicates. Results were calculated and expressed in terms of % RSD.

2.4.3 Repeatability

Repeatability of measurement of absorbance in UV was carried out using six replicates of same drug concentration. Absorbance was measured six times and % RSD was calculated.

Repeatability of measurement of peak area in HPTLC was carried out using six replicates of same spot. These were analyzed by spotting 8 ng of standard drug solution on a precoated silica gel plate, followed by development of plate and recording the peak area after each scanning (6 times) without adjusting the position of plate and % RSD was calculated.

2.5 Analysis of formulation

Twenty capsules of Loxapine each containing 25mg/ capsule were taken and average mean was calculated. They were pulverized to fine powder in a glass mortar and pestle. The quantity of powder equivalent to 10 mg was transformed accurately to 10 ml volumetric flask. It was dissolved by adding water (for UV) and methanol (for HPTLC). After sonication, the volume was made up to 10 ml using respective solvent. They were further diluted and analyzed by the developed UV and HPTLC methods.

Accuracy: Accuracy of the method was evaluated by standard addition technique. To the preanalyzed formulation standard Loxapine was added at 50% and 100% levels. The mixture was reanalyzed by the two methods and % accuracy was calculated.

3. RESULTS AND DISCUSSION

UV spectroscopy and HPTLC methods were developed and validated according to the experimental conditions of the study. The UV spectrum of Loxapine is shown in figure 2. A typical densitogram of Loxapine is shown in figure 3.

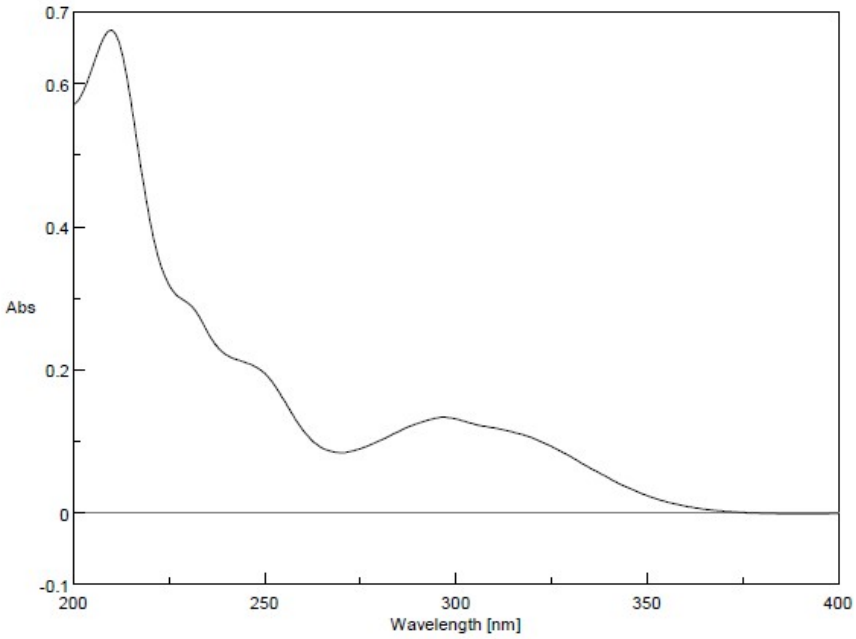

Fig. 2: UV absorbance spectra of Loxapine

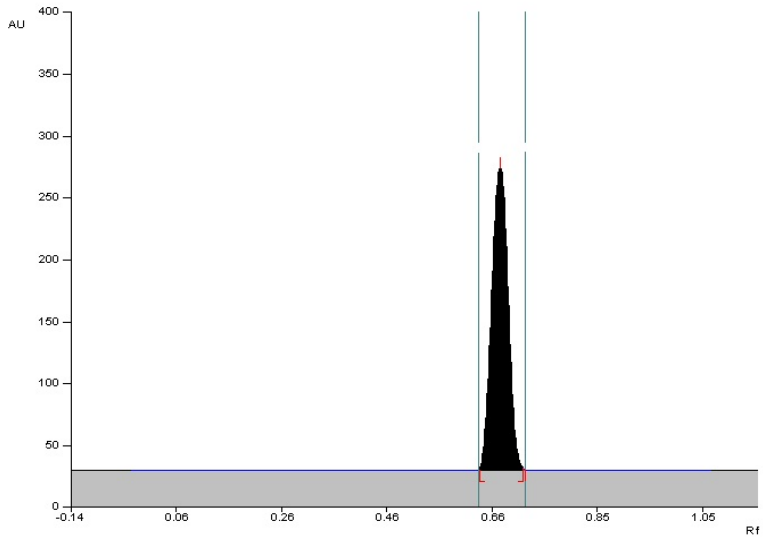

Fig.3: Densitogram of Loxapine

Linearity was found over the concentration range of 10- 80 µg/ml for UV method and 2– 12 ng/spot for HPTLC method with respective correlation co efficient 0.9991 and 0.9933 respectively. The calibration data and standard curves for Loxapine are shown in table 1 and figure 4 and 5.

Table 1: Calibration data for UV and HPTLC

Parameters	UV	HPTLC
Linearity range	10 – 80 µg/ml	2 - 12 ng/ml
Correlation coefficient (r)	0.9991	0.9933

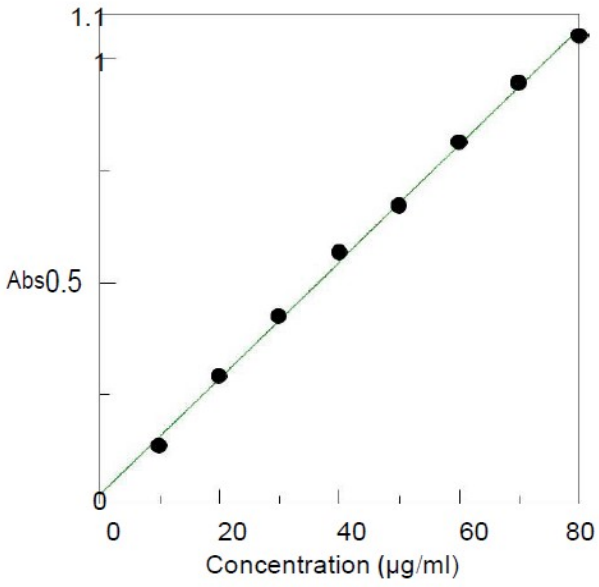

Fig.4: Standard graph of Loxapine obtained by UV spectroscopic method

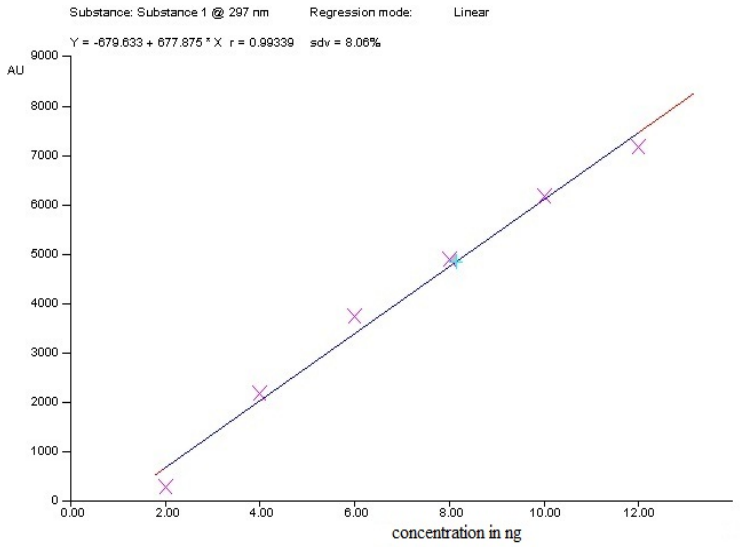

Fig.5: Standard graph of Loxapine obtained by HPTLC

Intra and inter day precision studies were carried out and the results are tabulated in table no: 2 and 3 for the two methods developed.

Table 2: Intraday and Interday precision for UV spectroscopic method

Concentration (µg/ml)	Absorbance		% RSD	
	Intraday	Interday	Intraday	Interday
40	0.5636	0.5611	0.65	0.71
	0.5620	0.5684		
	0.5691	0.5679		
50	0.6688	0.6680	0.10	0.51
	0.6673	0.6645		
	0.6682	0.6612		

Table 3: Intraday and Interday precision for HPTLC method

Concentration (ng/spot)	Peak area		% RSD	
	Intraday	Interday	Intraday	Interday
6	4087.4	4065.2	**0.80**	**0.28**
	4073.1	4066.7		
	4025.3	4046.3		
8	5054.8	5031.2	**0.15**	**0.50**
	5047.5	5028.9		
	5039.6	5073.8		

Repeatability of measurement for UV spectroscopic and HPTLC methods were carried out by repeating the procedure six times with the same concentration and the results are tabulated in table 4 and 5.

Table 4: Repeatability of measurement for UV spectroscopic method

Concentration (µg/ml)	Absorbance	% RSD
50	0.6688	0.34
	0.6692	
	0.6654	
	0.6643	
	0.6637	
	0.6659	

Table 5: Repeatability of measurement for HPTLC method

Concentration (ng/spot)	Peak area	% RSD
8	5054.8	0.37
	5039.5	
	5061.8	
	5045.9	
	5028.1	
	5010.2	

Analysis of Loxapine in formulation studies were performed and the amount of Loxapine present was calculated and presented in table 6.

Table 6: Analysis of formulation by UV and HPTLC methods

Trade name	Labeled claim mg/capsule	Calculated labeled claim mg/capsule		% label claim		% RSD*	
Loxapine	10	**UV**	**HPTLC**	**UV**	**HPTLC**	**UV**	**HPTLC**
		9.893	9.971	98.75	99.62	0.58	0.41

** An average of 6 determinations*

Standard addition technique was used to evaluate the accuracy of the two methods and % accuracy was calculated and shown in table 7.

Table 7: Recovery study

Level	% Recovery		% RSD	
	UV	**HPTLC**	**UV**	**HPTLC**
50%	99.78	100.20	0.21	0.49
100%	98.83	100.58	0.45	0.25

Repeatability, Intraday and Interday studies shows % RSD less than 2, indicating the precision of the developed method. The Loxapine solution was found stable for 7 hrs in room temperature and 18 hrs in refrigeration. The drug on developed plates was stable for 9 hours. These methods are more sensitive and cost effective. Hence, they can be employed for the quantitative determination of capsules containing Loxapine and % label claim was satisfactory.

4. CONCLUSION

The developed UV and HPTLC analytical methods are simple, rapid, accurate, precise and cost effective for the estimation of Loxapine. As there are no UV and HPTLC methods developed for Loxapine, they can be used for rapid analysis of Loxapine with reduced runtime and routine analysis of Loxapine in bulk and capsule dosage forms and quality control analysis.

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