International Journal of Advances in Pharmaceutical Analysis
IJAPA Vol. 5 Issue 3 (2015) 61-64

Development and validation of RP-HPLC method for loxapine in capsule dosage form

Dammalapati Srikantha and Rudraraju Rameshraju*

Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar-522 510, Guntur, Andhra Pradesh

Abstract

A reversed phase-high performance liquid chromatography (RP-HPLC) method was developed for the determination of Loxapine drug in tablet dosage form. The developed method was validated by measuring linearity, precision, limit of detection (LOD), robustness and ruggedness, drug recovery and for system suitability. Water and acetonitrile in the ratio 55:45 v/v was the mobile phase, C_{18} column (250 mm x 4.6 mm x 5 µm) as a stationary phase, and 265 nm was the detection wavelength. The HPLC system was operated in isocratic mode. The measured retention time of Loxapine drug was 6.88 minutes and the limit of detection was 0.035 µg/ml, respectively. The linearity measured in the range 10-100 µg/ml had a correlation coefficient of 0.999. The results of the parameters precision, robustness, recovery and formulation assay indicate that the developed method is a very good tool for the analysis of Loxapine drug in bulk and capsule dosage form.

Keywords: Loxapine; RP-HPLC; UV detection; Retention time; Tablet dosage form.

1. Introduction

Loxapine, a member of the dibenzoxazepine class, is an antipsychotic drug mainly used for the treatment of schizophrenia [1]. Its systematic (IUPAC) name is 2-chloro-11-(4-methylpiperazin-1-yl)dibenzo[b,f][1,4]oxazepine and the chemical formula is $C_{18}H_{18}ClN_3O$ [2, 3]. The Loxapine molecular structure is shown in Fig. 1 and its molecular weight is 327.81 g/mol [2, 3].

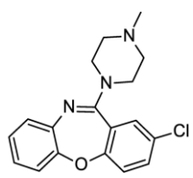

Fig. 1: The chemical structure of Loxapine.

Loxapine was investigated alone in plasma [4, 5], human plasma [6, 7] and in combination with other drugs [8-15]. It was also studied with other drugs in plasma [8], human plasma [9], with its metabolites in plasma [10], human plasma [11, 12], rat plasma [13], and serum and urine [14]. Various analytical techniques have been employed to determine Loxapine. They were high performance liquid chromatography (HPLC) [4, 6-8, 10-11], HPLC - MS/MS [9], HPLC – MS [12], Liquid Chromatography (LC)-MS/MS method [13] gas liquid chromatography (GLC) [14] and capillary zone electrophoresis [15].

In this paper, we have developed a method for Loxapine in capsule dosage form and validated it following the ICH guidelines [16, 17] with the parameters linearity, limit of detection, precision, robustness, ruggedness and system stability. The developed method is fast, cost effective because easily available chemicals are used as mobile phase and can be used for the analysis of commercially available drugs both in laboratories and industries.

2. Materials and methods

The chemicals and reagents used for the present study were of HPLC grade. Methanol, water (pH between 5 and 8) and acetonitrile were purchased from Merck Specialties Private Ltd., Mumbai, India.

A PEAK HPLC system operated in isocratic mode was employed for the reported work. It was equipped with a LC 20AT pump and a variable wavelength programmable ultraviolet (UV)-Visible detector (SPD-10AVP). As a stationary phase, Chromosil C_{18} column (250 x 4.6 mm, 5 µm particle size) was used. The samples were injected with a 20 µL Hamilton syringe. Degassing of the mobile phase was carried out using an ultrasonic bath sonicator (Loba). Weighing of the samples was done with a Denver (SI234) balance. Chromatograms were recorded and integrated using PEAK software. The obtained data was analyzed using Microsoft Excel. The absorption wavelength of Loxapine was determined using an ultraviolet (UV) - Visible spectrophotometer (Techcomp UV 230D6) with HITACHI software.

2.1 Preparation of stock solution

The stock solution for the present study was prepared from a 10 mg of Loxapine drug. After weighing it was dissolved in 10 ml of mobile phase in a 10 ml volumetric flask. The solution was sonicated for 2 minutes to dissolve the drug completely. After cooling, it was filtered with a 0.45 µm nylon membrane ultipore filter paper.

A 1000 µg/ml stock solution was prepared and from this, 2 ml was further diluted to 20 ml and obtained a stock concentration of 100 µg/ml solution. By selective dilution, required concentrations were prepared from the stock solution.

2.2. Method development

The RP-HPLC conditions like mobile phase composition, flow rate, wavelength were optimized for a sharper chromatogram peak and to fulfill the ICH guide lines. Firstly, mobile phase volume ratio was developed. In general, the mobile phase will be chosen by controlled trial and error method. This mainly depends on the suitability to the drug sample to be analyzed, cost-effectiveness and from the information available from the literature. Here, standard organic solvents methanol, acetonitrile and water were tested separately and in combination as a mobile phase. From the observation, water and acetonitrile combination showed better results. Then different volume ratios of water and acetonitrile were tried and finally the 55: 45 v/v gave a sharper chromatogram, high theoretical plates and low tailoring factor. Thus, we choose this as optimal mobile phase for the present study. After several iterations (trials) and chromatographic runs, pH of 6.6 with addition sodium acetate resulted in a better peak symmetry and good signal to noise (S/N) ratio.

The active pharmaceutical ingredient (API) concentration chosen was 60 µg/ml. This was also used as standard concentration as it was the optimum concentration

Corresponding Author*: rrraju1@gmail.com

from the Beer Lambert's law obtained from the linearity range measurements. The pump pressure was noted down during the development phase and the optimum value was 7.5 MPa for the standard solution. The flow rate used for the reported results was 0.8 ml/min.

The run time was selected after checking for the interferences from the excipients. The optimum conditions obtained during the method development and used for validation of various parameters were: mobile phase - water: acetonitrile (55: 45 v/v); detection wavelength - 265 nm; stationary phase column - C_{18} column (250 mm x 4.6 mm, 5 µm); pH of the mobile phase - 6.6 with sodium acetate; API concentration - 60 µg/ml; flow rate - 0.8 ml/min; pump pressure - 7.5 ± 0.5 Mpa and runtime - 10 minutes.

3. Results and discussion

In order to determine the best wavelength of maximum absorption of Loxapine spectrophotometric method was used. The wavelength was scanned in the range of 200 nm - 400 nm. The maximum absorption was observed at 265 nm (Fig. 2).

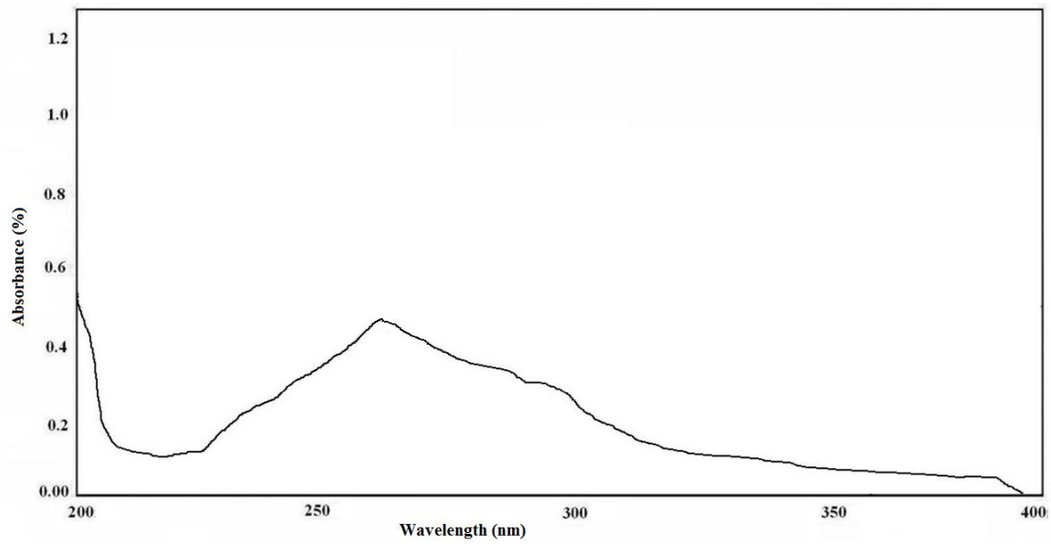

Figure 2: Absorption spectrum of loxapine with wavelength (nm) vs. absorbance (%).

The parameters described below have been validated for the developed method in accordance with the International Conference and Harmonization (ICH) guidelines [13, 14]. The general acceptance criteria is that residual standard deviation (RSD) of peak areas should be less than 2%. Further the system suitability was evaluated from the theoretical plate number and tailing factor. The theoretical plate (TP) numbers should be at least 2500 for each peak and it was 20345 for the standard solution, a factor of 8.1 more than the recommended value. The tailing factor value has to be less than 2, which was 1.12 for the presented results. In Fig. 3 shown was the chromatogram of Loxapine obtained for the standard solution with optimized conditions.

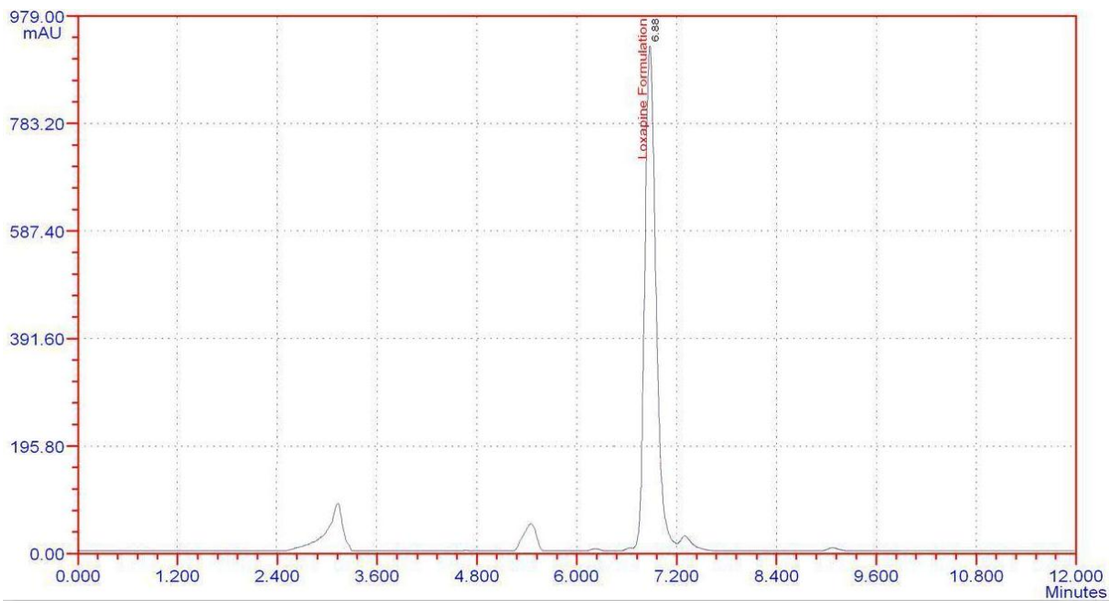

Figure 3: Chromatogram of Loxapine from formulation assay. The retention time is 6.88 minutes.

3.1 Linearity

The linearity of the peak areas was determined for 10 different concentrations of Loxapine in the 10-100 µg/ml range. The calibration graph was obtained by plotting peak areas as ordinates and the corresponding ten concentrations (µg/ml) as abscissa (fig. 4). The linear regression y = mx + c, where 'm' the slope of the line and 'c' the y-intercept in the fit to the data. The linear regression was found to be precise from the correlation coefficient, $R^2 = 0.999$.

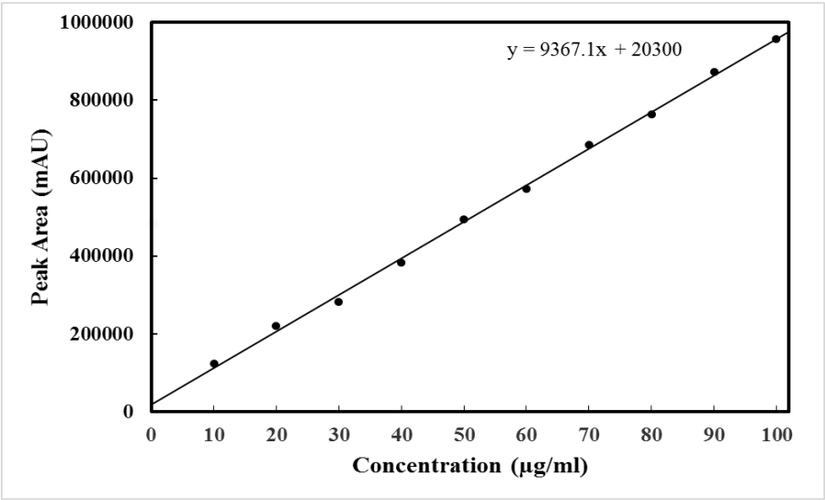

Fig. 4: Linearity results of Loxapine.

3.2 Precision

A widely validated parameter for drugs was the repeatability for over a period of time through intra-day (same day) and inter-day (different days) precision studies. Here, the intra-day precision as well as inter-day precision was studied at 60 µg/ml of Loxapine (Table 1). The data given in Table 1 from intra-day precision and inter-day precision were mean normalized area. The mean given was obtained from the normalized area of the samples. The normalization was done with respect to the area of one of the six (n=6) samples. Also, given in Table 1 were standard deviation (SD) and relative standard deviation (RSD) of the precision measurements. The RSD of the samples for both intra-day (1.31) and inter-day (1.73) measurements conclude that the method was precise.

Table 1: Results of intra-day, inter-day precision and ruggedness for Loxapine.

Parameter	Concentration (µg/ml) (n=6)	Normalized Area Mean ± SD	RSD
Intra-day precision	60	1.011 ± 0.013	1.31
Inter-day precision	60	1.008 ± 0.018	1.73
Ruggedness	60	0.989 ± 0.178	1.79

3.3 Ruggedness

Loxapine concentration of 60 µg/ml was also used to study the ruggedness of the method. In the present work, this was verified by two different analysts for the same concentration of Loxapine. Six (n = 6) samples in total were analyzed. The effect of these changes on the peak area was evaluated by calculating the RSD. The results were given Table 1. The RSD value obtained was less than 2% indicating the ruggedness of the procedure.

3.4 Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection and limit of quantification play an important role in determining the sensitivity of the method developed for the studied sample. Detection and quantification limits were calculated from the calibration equations obtained from the experiment. This was based on the signal to noise (S/N) ratio, repeatability and system suitability. The lowest concentration where the S/N ratio was better was chosen as the limit of detection (LOD). Then LOQ was determined from the equation, LOQ = 3.3 LOD. For Loxapine, the values for limit of detection was 0.035 µg/ml and the limit of quantification was 0.1 µg/ml, respectively.

3.5 Robustness

The robustness of the developed method was very important in determining the effects of variations to the instrumental parameters. This was done by deliberately changing the mobile phase volume ratio (<10%), pH of the solution (10%) and detection wavelength (<1%). The changes observed in the peak areas with respect to the peak area of the optimized conditions were given in Table 2.

Table 2: Results of robustness data of Loxapine for three parameters

Parameter	Change	Peak Area (mAU)	% of change in peak area RSD
Standard	No change	569851	---
Mobile phase Water : Acetonitrile	60 : 40 v/v	580195	1.81
	50 : 50 v/v	572066	0.38
pH	6.5	576337	1.13
	6.7	574355	0.79
Wavelength	260 nm	562069	-1.36
	270 nm	579996	1.78

The percentage change of peak areas was calculated for each changed parameter and was found to be less than 2% (Table 2). The method was robust at 10% variation of the mobile phase composition and about a factor of 5 changes in peak areas was observed between the changed concentrations of mobile phase. The change in the pH of the solution resulted in negligible changes in the peak area. About a percent decrease in wavelength from the central value showed decrease in the peak area. But, a percentage increase in wavelength from the central wavelength value showed increase in the peak area. This proved to be a sensitive parameter. The above measurements indicate robustness of the developed method.

3.6 Recovery

To check for the accuracy of the proposed method, recovery experiments were carried out by standard addition technique by adding a known amount of standard at three different levels (50%, 100% and 150%) to the sample. The analysis of each level was repeated three times (n = 3). The results were given in Table 3. For example, if the target amount was 20 µg/ml, 50% of the target solution was spiked with 10 µg/ml solution making the total concentration as 30 µg/ml. Now area of the peak 40 µg/ml is compared with the standard 60 µg/ml solution peak. Then the two areas are compared, from which concentration was measured for the percentage recovery (Table 3). Finally, the percentage recovery of Loxapine was compared with the actual amounts. The good recovery of the product in the range of 99.7% to 100.18% suggests the high accuracy of the method. The percentage error for recovery was given in last column.

Table 3: Recovery study results of loxapine at three percentage levels.

% added	Target concentration (µg/ml) (n=3)	Spiked concentration (µg/ml)	Final concentration (µg/ml)	Concentration Obtained Mean ± SD	RSD or CV (%)	Recovery (%) Mean ± SD	Error [a](%)
50%	20	10	30	30.05 ± 0.38	1.27	100.18 ± 1.28	0.74
100%	20	20	40	40.06 ± 0.64	1.58	100.15 ± 1.59	0.92
150%	20	30	50	49.85 ± 0.48	0.95	99.7 ± 0.95	0.55

a % Error = RSD/√n. No. of trails, n = 3

3.7 Formulation assay

The assay of the proposed method was applied to Loxapine available in commercial tablets in fixed dosage form (Loxapac). Twenty capsules of Loxapac tablets were weighed and powdered. The average weight of the powder was 88 mg. The capsule powder equal to 10 mg of the drug was dissolved in 100 ml of methanol. From the concentration of 100 µg/ml solution, 60 µg/ml was prepared and used for formulation assay studies. Assay results of Loxapine expressed as a percentage of label claim was in good agreement within 90 to 100% of the label claim (Table 4). In the chromatogram only one dominant peak was observed. There was no interference from other excipients and their peaks were negligible (Fig. 3).

Table 4: Formulation assay of Loxapine

Formulation	Form	Dosage	Concentration	Amount found	% Assay
Loxapac	Capsule	50 mg	60 µg/ml	59.46	99.09

4. Conclusion

In conclusion, Loxapine was determined in capsule dosage form using RP-HPLC method. The developed method provided a simple, precise and accurate way for the determination of Loxapine. The developed method facilitates faster analysis of Loxapine with reduced runtime and the statistical analysis of the parameters prove that the method provides good recovery, robustness and precision. The method and the systems were suitable for routine analysis of Loxapine in capsule dosage form.

References

[1] Heel RC *et al*. Loxapine: a review of its pharmacological properties and therapeutic efficacy as an antipsychotic agent. *Drugs* 1978; 15(3): 198-217.

[2] Martindale The Extra Pharmacopoeia, 31st ed, Royal Pharmaceutical Society of Great Britain, London, 1996.

[3] US Pharmacopeia, 23rd ed, United States Pharmacopeial Convention, Rockville, MD, 1995.

[4] Yu-guan W, Ri-fang L, Shao-fang C. Determination of loxapine in plasma by HPLC. Chinese *J Hospital Pharm* 2006; 26 (10): 1234.

[5] Wen Yu-guan *et al.* Rapid determination of loxapine in plasma and clinical application. *Pharmacy Today* 2008; 2: 024.

[6] Ao Z, Wei-giao H, Qing-xia C, Zhong W. Determination of loxapine in human plasma by RP-HPLC. *Central South Pharmacy* 2009; 6: 10.

[7] Ao Z, Wei-giao H, Qing-xia C, Zhong W. Determination of the concentration of loxapine in human plasma by HPLC with UV detection. *Strait Pharm J* 2009; 6: 112.

[8] Garcia LG, Bares IF, Pehourcq F, Jarry C. Simultaneous determination of four antipsychotic drugs in plasma by high performance liquid chromatography: Application to management of acute intoxications. *J Chromatogr* B 2003; 795(2): 257-264.

[9] Zimmer JSD, Needham SR, Christianson CD *et al.* Validation of HPLC-MS/MS methods for analysis of loxapine, amoxapine, 7-OH-loxapine, 8-OH-loxapine and loxapine N-oxide in human plasma. *Bioanalysis* 2010; 2(12): 1989-2000.

[10] Cheng SW, Tang SW, Remington G. Simultaneous quantitation of loxapine, amoxapine and their 7- and 8-hydroxy metabolites in plasma by high performance liquid chromatography. *J Chromatogr* 1991; 564(1): 213-21.

[11] Mercolini L, Mandrioli R, Finizio G, Boncompagni G, Raggi MA. Simultaneous HPLC determination of 14 tricyclic antidepressants and metabolites in human plasma. *J sep sci* 2010; 33(1): 23-30.

[12] Choong E *et al.* Therapeutic drug monitoring of seven psychotropic drugs and four metabolites in human plasma by HPLC-MS. *J Pharm and Biomed anal* 2009; 50 (5): 1000-1008.

[13] Wong YC, Wo SK, Zuo Z. Investigation of disposition of loxapine, amoxapine and their hydroxylated metabolites in different brain regions, CSF and plasma of rat by LC-MS/MS. *J Pharm and Biomed anal* 2012; 58: 83-93.

[14] Cooper TB and Kelly RG. GLC analysis of loxapine, amoxapine and their metabolites in serum and urine. *J Pharm sci* 1979; 68(2): 216-219.

[15] Pucci V, Raggi M and Kenndler E. Separation of antipsychotic drugs (clozapine, loxapine) and their metabolites by capillary zone electrophoresis. *J Chromatogr* A 1999; 853(1-2): 461-468.

[16] ICH Harmonized Tripartite Guideline Q2 (R1), Validation of analytical procedures: text and methodology, Current Step 4 Version, 2005.

[17] Ohannesian L and Streeter AJ, Hand book of Pharmaceutical analysis, Marcel Dekker Inc. NewYork; 2002.