Research Article

Stability Indicating Method Development and Validation for the Estimation of Lofepramine in Bulk and Marketed Tablet Dosage Form by RP-HPLC

Juveriya Fatima Siddiqui*, Asma Ibrahim, Farheen Begum, Mariam Ali, Mohd.Sohaib
Deccan School of Pharmacy, Department of Pharmaceutical Analysis, Hyderabad, Telangana, 500001, India.
***Corresponding author's E-mail:** juveriyafatimasiddiqui@gmail.com

Received: 16-04-2024; **Revised:** 26-06-2024; **Accepted:** 03-07-2024; **Published on:** 15-07-2024.

ABSTRACT

Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form of Lofepramine.

Methods: A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Lofepramine. The chromatographic strategy utilized Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm, using isocratic elution with a mobile phase of Phosphate Buffer (0.02M) and Acetonitrile were consisting of 48:52% v/v (pH-2.80). A flow rate of 1.0 ml/min and a detector wavelength of 248 nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.

Results: LOD and LOQ for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness was determined as a part of method validation and the results were found to be within the acceptable range.

Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drug.

Keywords: Lofepramine, RP-HPLC, Method Development, Validation, Accuracy, Robustness.

INTRODUCTION

According to a recent report by the World Health Organization, 56 million, i.e., 4.5% of Indians suffer from depression and another 38 million i.e., 3.5% Indians suffer from anxiety disorders.

Therapeutic drug monitoring (TDM) of antidepressants is necessary for an optimal supervision of patient drug regimen to avoid medical complications, intoxication, nonresponsiveness or noncompliance Pharmacological treatment for depression has advanced greatly since the development of the first therapies in the 1950s, with the introduction of monoamine oxidase inhibitors and tricyclic antidepressants (TCAs) Since the late 1980s, a whole new generation of chemically and neuropharmacologically unrelated agents has been introduced. These drugs appear to be safer and better tolerated and include: selective serotonin reuptake inhibitors (SSRIs) noradrenergic and specific serotonergic antidepressants reversive and selective monoamino oxidase inhibitor and a potent and balanced inhibitor of both serotonin and norepinephrine reuptake.[1-3]

Lofepramine

Lofepramine is a dibenzoazepine, a member of monochlorobenzenes, a tertiary amino compound and an aromatic ketone. It has a role as an antidepressant. A psychotropic IMIPRAMINE derivative that acts as a tricyclic antidepressant and possesses few anticholinergic properties. It is metabolized to desipramine.[4-6]

Review of literature for Lofepramine gave information regarding its physical and chemical properties, various methods that were conducted alone and in combination with other drugs. Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Lofepramine and single method is available for such estimation by RP-HPLC. Validation is a necessary and important step in both framing and documenting the capabilities of the developed method. The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.[18-24]

MATERIALS AND METHODS

Chemicals and Reagents

The reference standards were procured from Vivan Life Sciences, Hyderabad and the tablet dosage form was purchased from the local pharmacy. HPLC grade water and acetonitrile were purchased from Bhiwandi, Maharashtra and analytical grade chemicals such as Potassium

dihydrogen phosphate, Orthophosphoric acid were purchased from E. Merck limited, Mumbai.

Method Development

Separation was performed using Symmetry ODS (c_{18}) RP column, 250 mm x 4.6 mm, 5µm with Mobile Phase Was Phosphate Buffer (0.02m): Acetonitrile = 48:52 (ph-2.80). The samples were analysed using 20µl injection volume maintaining the flowrate 1.0 ml/ min with a run time of 8 min and the temperature was maintained at ambient condition. Detection was achieved using PDA detector at 248 NM wavelength and the retention time was found to be 3.867 minutes.

Mobile Phase

Mobile Phase for the optimized trial was Phosphate Buffer (0.02m): Acetonitrile in the ratio of 48:52.

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Lofepramine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.5ml of the above Lofepramine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Sample Solution:

Twenty tablets were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Lofepramine equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.5 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

RESULTS AND DISCUSSIONS

1. Specificity:

Capacity of the method to measure the Analyte peak response in the presence of the other components is termed as Specificity. Specificity can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. The chromatogram for the blank is given in the figure 2. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific[16].

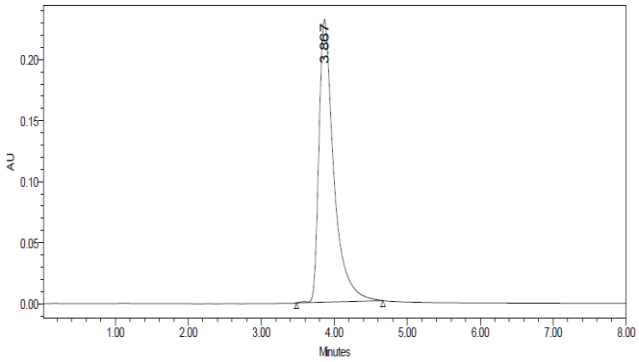

Figure 1: Optimized chromatogram of the proposed method

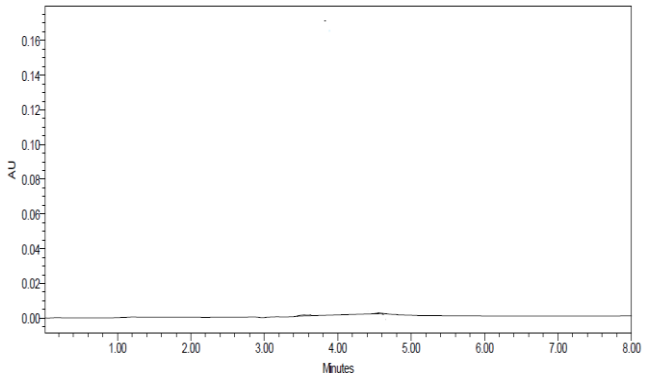

Figure 2: Chromatogram for Blank Solution

2. System Suitability Parameters:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.[15] The results for system suitability of Lofepramine is given in the table 1.

3. Linearity and Range:

An appropriate volume of aliquots from standard Lofepramine stock solution were transferred to different volumetric flasks. The volumes were adjusted to the mark with diluent to give a solution containing concentration of 30, 40, 50, 60, 70 µg/ml of Lofepramine. The linearity graphs for Lofepramine are shown in figure 3.[12-13]

4. Precision:

The method precision(repeatability) was performed by carrying out six independent assays of the test sample of 50 µg/ml of Lofepramine. The intermediate precision was evaluated by carrying out six independent assays of test samples by different analysts at 50µg/ml. The data for repeatability and intermediate precision is shown in the table no 2 and 3,4 respectively.

Table 1: Results of System Suitability for Lofepramine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Lofepramine	3.644	584635	65847	4857	1.48
2	Lofepramine	3.645	582695	65421	4955	1.42
3	Lofepramine	3.644	587432	65369	4875	1.47
4	Lofepramine	3.662	589687	65748	4796	1.46
5	Lofepramine	3.660	582547	65398	4952	1.49
6	Lofepramine	3.660	589656	652418	4896	1.47
Mean			**586108.7**			
Std.Dev.			**3275.654**			
%RSD			**0.558882**			

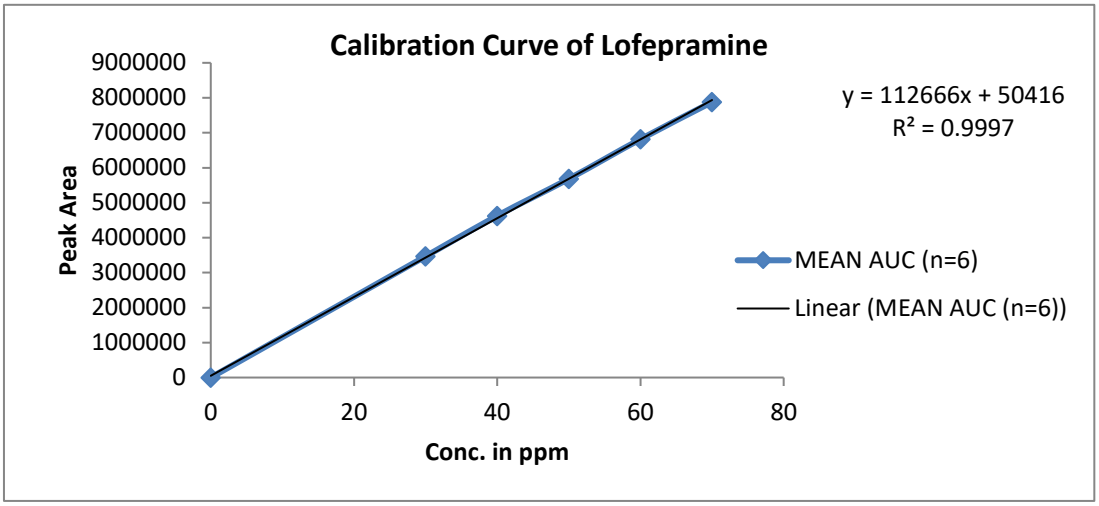

Figure 3: Linearity graph for lofepramine

Table 2: Repeatability Readings

HPLC Injection Replicates of Lofepramine	Retention Time (Minutes)	Peak Area
Replicate – 1	3.649	**5674158**
Replicate – 2	3.684	**5654715**
Replicate – 3	3.687	**5665841**
Replicate – 4	3.688	**5654578**
Replicate – 5	3.688	**5652284**
Replicate – 6	3.687	**5641487**
Average		**5657177**
Standard Deviation		**11369.72**
% RSD		**0.200979**

Table 3: Results of Ruggedness for Lofepramine Analyst 1

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	Plate Count	Tailing
1	Lofepramine	3.687	584968	65982	4985	1.42
2	Lofepramine	3.688	582479	66354	4876	1.46
3	Lofepramine	3.688	586236	67425	4896	1.48
4	Lofepramine	3.687	586985	65982	4986	1.47
5	Lofepramine	3.684	582679	65932	5016	1.45
6	Lofepramine	3.649	583989	65874	4987	1.43
Mean			**584556**			
Std.Dev.			**1846.658**			
%RSD			**0.315908**			

Table 4: Results of Intermediate Precision Analyst 2 for Lofepramine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	Plate Count	Tailing
1	Lofepramine	3.649	598698	66985	5265	1.49
2	Lofepramine	3.684	596847	67458	5168	1.47
3	Lofepramine	3.687	596354	66985	5436	1.46
4	Lofepramine	3.688	598676	67854	5369	1.45
5	Lofepramine	3.688	596874	68521	5247	1.48
6	Lofepramine	3.687	598989	67898	5375	1.42
Mean			**597739.7**			
Std.Dev.			**1168.098**			
%RSD			**0.195419**			

Table 5: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Mean
	Amount Added	Amount Found			
S_1 : 80 %	40	40.141	502647	100.352	100.3947%
S_2 : 80 %	40	40.191	503214	100.477	
S_3 : 80 %	40	40.142	502656	100.355	
S_4 : 100 %	50	50.044	614215	100.088	99.98533%
S_5 : 100 %	50	49.887	612451	99.774	
S_6 : 100 %	50	50.047	614254	100.094	
S_7 : 120 %	60	60.192	728547	100.32	100.311%
S_8 : 120 %	60	59.939	725698	99.898	
S_9 : 120 %	60	60.429	731211	100.715	

Table 6: Results for Robustness

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	584624	3.649	1.42	4765
Less Flow rate of 0.9 mL/min	598676	3.687	1.49	4856
More Flow rate of 1.1 mL/min	612543	3.649	1.46	4965
Less organic phase	578642	3.688	1.49	4758
More organic phase	569896	3.684	1.47	4962

5. Accuracy:

To determine the accuracy of the planned technique, recovery studies were distributed by adding completely different amounts (80%, 100%, and 120%) of pure drug of Lofepramine were taken and extra to the pre-analyzed formulation of concentration 50µg/ml. From that proportion recovery values were calculated. The results were shown in table 5.[7-9]

6. Method robustness:

The robustness study was performed by slight modifications in the flow rate and by mobile phase ratio variation from more organic phase to less organic phase ratio for Lofepramine.

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. The data for robustness is shown in the table 6.[14]

7. LOD & LOQ: The LOD and LOQ of the developed method was calculated based on the standard deviation of the response and the slope of the linearity curve. The LOD was found to be 0.33µg/ml and LOQ was found to be 1.009 50µg/ml.

Analysis of the tablets

The proposed method was used for the assay of commercially available tablets of lofepramine. The assay was performed in triplicates. The assay percentage of lofepramine was found to be within limits of 99.56%.

Stability studies:

1. Acidic Degradation: Accurately weigh 10 mg of Lofepramine was added to a clean and dry volumetric flask. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water bath at 60°C for 4 hours. Allow it to cool to room temperature. The sample was then neutralized using dilute NaOH solution & final volume of the sample was made up to 100ml with water to prepare 100 µg/ml solution. It was introduced into the HPLC framework against a clear mobile phase. This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile.

2. Basic Hydrolysis: Accurately weigh 10 mg of Lofepramine was added to a clean and dry volumetric flask. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water bath at 60°C for 4 hours. Allowed to cool to room temperature. The sample was then neutralized using 2N HCl arrangement and last volume of the sample was made up to 100ml to plan 100 µg/ml solution. It was introduced into the HPLC framework against a clear of mobile phase with the aim of enhancing the versatile stage arrangements. This experiment was repeated several times using same concentration of NaOH such as 0.1N to observe its degradation profile.

3. Thermal Degradation: Accurately weigh 10 mg of Lofepramine was added to a clean and dry volumetric flask and 30 ml of HPLC water was added to it. Then, it was refluxed in a water bath at 60° c for 6 hours uninterruptedly. After the reflux was over, the drug became soluble and the mixture of drug & water was allowed to cool to room temperature. Last volume was made up to 100 ml with HPLC water to prepare 100 µg/ml solution. It was introduced into the HPLC framework against a clear of mobile phase.

4. Photolytic Degradation: Accurately weigh 10 mg of Lofepramine was added to a clean and dry Petri dish. It was kept in an UV spectrophotometer at 254 nm wavelength for 24 hours without interference. Accurately measured 1 mg of the UV uncovered Lofepramine was added to a clean and dry 10 ml volumetric cup. First the UV exposed drug was dissolved in methanol & made up to the mark with mobile phase to get 100 µg/ml solution. At last, this solution was infused into the HPLC framework against a clear of mobile phase and chromatogram was obtained.

5. Oxidation with (3%) H_2O_2: Accurately weigh 10 mg of Lofepramine added to a clean and dry 100ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it dissolvable and then kept in that capacity in dark for 24 hours. Last volume was made up to 100 ml using water to get ready 100 µg/ml solution. The above example was infused into the HPLC framework.

Results of Degradation Studies: The results of the stress studies indicated the Specificity of the method that has been developed. Lofepramine was stable in photolytic and peroxide stress conditions. The result of forced degradation studies are given in the following table 7.

Table 7: Results of Forced Degradation Studies of Lofepramine API

Stress conditions	Time	Assay of active substance	Assay of degraded product	Mass balance (%)
Acid hydrolysis (0.1M HCL)	24 hrs	98.76	1.24	100.0
Basic hydrolysis (0.1 M NaOH)	24 hrs	98.63	1.37	100.0
Thermal Degradation	24 hrs	93.98	6.07	100.0
UV 248 nm	24 hrs	98.84	1.16	100.0
3% Hydrogen Peroxide	24 hrs	94.61	5.39	100.0

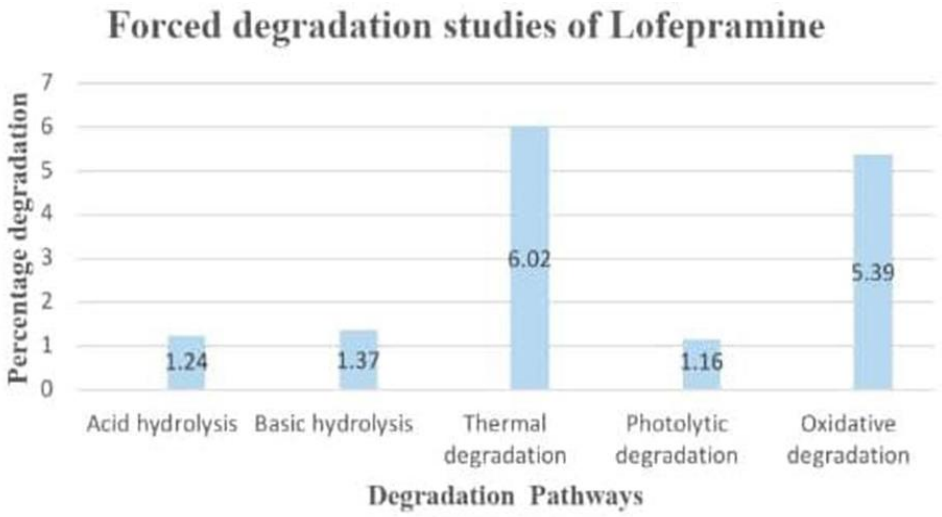

Figure 4: Bar graph representing data for % degradation.

CONCLUSION

The proposed HPLC method developed and validated as per the ICH guidelines and found to be applicable for routine quantitative analysis of Lofepramine by HPLC in bulk drug and in pharmaceutical dosage form. The Results of Linearity, Accuracy, Precision, Specificity, LOD and LOQ were proved to be within limits. The proposed method was highly Reproducible, Reliable, rapid, robust and specific. Therefore, a high percentage of recovery and a run time of less than 10 mins allows its application in its routine determination of Lofepramine in the pharmaceutical dosage form.

Acknowledgement: Our first salutation goes to almighty and our parents for being courteous and kind to us. I am grateful to Prof. Dr. Syed Abdul Azeez Honorable Principal Deccan School of Pharmacy Hyderabad for providing facilities to do this work and for his constant support and encouragement.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Tripathi K.D: Essentials of medical pharmacology, 5th edition, Medical publishers ltd, New Delhi, 2003,169-177.

2. Gurdeep R. Chatwal, Sham.K. Anand; Instrumental methods of chemical analysis; IJRR, 2020;18:673-9

3. Rabi Sankar, Instrumental Method of Analysis, 2021. P-18-6, P-18-3.

4. https://go.drugbank.com/drugs/DB13411

5. https://pubchem.ncbi.nlm.nih.gov/compound/Gamanil

6. https://en.wikipedia.org/wiki/Lofepramine

7. Guidance for industry, Analytical Procedure and Method Validation, U.S. Department of Health and Human Services FDA, August 2000.

8. ICH Q2B: Validation of Analytical Procedure; Methodology (International Conferences on Harmonization of Technical requirements for the registration of Drugs for Human use, Geneva, Switzerland, May 1997.

9. ICH Q2B: Validation of Analytical Procedure; Methodology (International Conferences on Harmonization of Technical requirements for the registration of Drugs for Human use, Geneva, Switzerland, Nov 2003.

10. Mohammad Tet al., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. International Pharmaceutical Sciences, 2012;2(3):14-21.

11. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012;2(4):22-23.

12. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.

13. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva.1996, 11. (CPMP/ICH/281/95).

14. Vibha Gupta et al, Development and validation of HPLC method - A Review, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.

15. Santosh Kumar Bhardwaj et al,A Review: HPLC Method Development and Validation, International Journal of Analytical and Bioanalytical Chemistry, 2015;2(1):1-25.

16. Lalit V Sonawan, Bioanalytical Method Validation and Its Pharmaceutical Application- A Review, Pharmaceutica Analytical Acta, 2014;5(3):80-86.

17. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. 2022.

18. Torben Elm, Ejvind Lyders Hansen, Simultaneous determination of Lofepramine and Desipramine by a High-Performance Liquid Chromatographic Method used for Therapeutic Drug Monitoring, Journal of Chromatography B: Biomedical Sciences and Applications, 1955;665(2):355-361.

19. Armagan Onal and Aysel Oztunc, A Rapid and Simple RP-HPLC Method for Quantification of Desipramine in Human Plasma, Reviews in Analytical Chemistry, 2011;30:165-169

20. Autumn R. Breaud, Robert Harlan, Marta Kozak, William Clarke, A rapid and reliable method for the quantitation of tricyclic antidepressants in serum using HPLC-MS/MS, Clinical Biochemistry, 2009;42(12):1300-1307.

21. Jinesh Bahubali Nagavi, Sunil Rajaram Dhaneshwar, Development and Validation of an RP-HPLC Method for the Determination of Stability Parameters for Clomipramine Hydrochloride, Indo American Journal of Pharmaceutical Research, 2013;4(4):1939-1948.

22. V.R.B. Vemula, P.K. Sharma,A method development and validation for simultaneous estimation of Imipramine and Diazepam in tablet dosage form by RP-HPLC; International Journal of Pharmacy and Pharmaceutical Sciences 2015;2013:249-253

23. P.P. Chauhan, D.Y. Patel,S.K. Shah, Optimization of stability indicating RP-HPLC method for the estimation of an antidepressant agents alprazolam and imipramine in pure & pharmaceutical dosage form, 2016, 200-206.

24. V Pavan Kumar, B Ramadevi; Development and validation of new analytical method for the simultaneous estimation of amitriptyline and perphenazine in bulk and pharmaceutical dosage form by RP-HPLC; International Journal of Research in Pharmaceutical Sciences and Technology, 2018;1(1):12-21.