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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LOFEPRAMINE HYDROCHLORIDE USING HPLC

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ABSTRACT

This study aimed to develop a reliable and reproducible analytical method for the quality control of Lofepramine hydrochloride by evaluating its physicochemical properties, analytical behavior, and stability. The solubility study indicated that Lofepramine hydrochloride is slightly soluble in water, ethanol, and 0.1 N HCl, but freely soluble in methanol and soluble in 0.1 N NaOH, which is critical for its dissolution and formulation strategies. A chromatographic method using a 50:50 mixture of acetonitrile and methanol at a flow rate of 1.0 mL/min was optimized, yielding well-resolved peaks and meeting system suitability parameters. Linearity studies showed a strong linear response (2 to 10 µg/mL) with excellent precision and accuracy. Recovery studies at 80%, 100%, and 120% levels demonstrated consistent results, confirming the method's accuracy. The method exhibited high precision, both intra-day and inter-day, with low % RSD values. The robustness study indicated the method's resilience to variations in temperature, flow rate, and mobile phase ratio, ensuring its reliability for routine quality control. Forced degradation studies revealed that Lofepramine hydrochloride is most stable under acidic conditions, with the highest degradation occurring under oxidative and photolytic stress. In conclusion, the developed analytical method for Lofepramine hydrochloride is accurate, precise, robust, and suitable for quality control and stability testing of pharmaceutical formulations, ensuring the consistency and efficacy of the drug product.

Keywords: Lofepramine hydrochloride, Chromatography, System suitability, Linearity, Recovery studies, Precision, Robustness, Forced degradation.

INTRODUCTION

Lofepramine hydrochloride is a tricyclic antidepressant primarily used in the treatment of depression and anxiety disorders. It is structurally related to other tricyclic antidepressants but with fewer side effects, making it an essential drug in psychiatric and neurological therapies (Sander *et al.,* 2014). Like many pharmaceutical compounds, Lofepramine hydrochloride must undergo rigorous testing to ensure its quality, efficacy, and safety in drug formulations. A critical aspect of this process is the development of reliable and reproducible analytical methods for its estimation, particularly to assess its purity, stability, and overall quality.

Pharmaceutical quality control plays a vital role in ensuring that drugs meet predefined standards for strength, purity, and composition. Among the different analytical

techniques, High-Performance Liquid Chromatography (HPLC) has emerged as the gold standard due to its high sensitivity, precision, and versatility in analyzing pharmaceutical substances in various forms (Shabir & Jamil, 2007). HPLC is particularly suited for the development of stability-indicating methods (SIM), which are essential for determining the stability of a drug under various stress conditions, such as temperature, light, oxidation, and hydrolysis (FDA, 2017). These methods are designed to differentiate between the active pharmaceutical ingredient (API) and its degradation products, ensuring the reliability and safety of the drug throughout its shelf life.

Lofepramine hydrochloride, being a complex organic compound, may undergo various degradation processes under different conditions, including acid and base hydrolysis, oxidation, and photolysis, which can impact its therapeutic efficacy (Sander *et al.,* 2014). Therefore, the development of a stability-indicating method (SIM) for Lofepramine hydrochloride is of utmost importance to assess its stability and ensure that degradation products do not compromise the quality of the drug. This method should be capable of detecting even low concentrations of degradation products, providing clear separation of the API and its degradation products.

A stability-indicating method should also undergo comprehensive validation to ensure it meets regulatory standards set by authorities such as the International Conference on Harmonization (ICH) (ICH, 2005). The validation process involves assessing parameters like specificity, linearity, accuracy, precision, robustness, and sensitivity to ensure the reliability and consistency of the analytical results across different conditions and over time.

Recent studies on the stability of pharmaceutical compounds have highlighted the importance of developing and validating analytical methods that can efficiently detect and quantify both the active ingredient and its degradation products. Various methods, including HPLC and other chromatographic techniques, have been explored for stability studies of antidepressants and other drugs in this class, emphasizing the need for effective quality control in pharmaceutical production (Shabir & Jamil, 2007; ICH, 2005).

In this study, we aim to develop and validate a stability-indicating method for the estimation of Lofepramine hydrochloride using HPLC. The method will be assessed for its ability to separate the drug from its degradation products, and it will be validated based on ICH guidelines to ensure its applicability for routine quality control in pharmaceutical formulations.

MATERIALS AND METHODS

Selection of mobile phase

Initially to estimate Lofepramine hydrochloride number of mobile phase in different ratio were tried. Results were shown in table.

Taking into thought the system suitability parameter like RT, Tailing factor, no. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Methanol and Acetonitrile in the ratio of 50:50v/v. The mobile phase was filtered through 0.45 µm filter paper to remove particulate matter and then degassed. Flow rate employed for analysis was 1.0 ml/min (Agrahari *et al.,* 2013).

Selection of wavelength

10 mg of Lofepramine hydrochloride was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the mobile phase. From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at λ_{max} 290.00 nm (Swartz *et al.,* 2010).

Selection of separation variable

Standard drug solution of Lofepramine hydrochloride was prepared in different mobile phase and chromatograph was recorded by using different column (5µm) at different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment (Shalliker *et al.,* 2008).

System suitability parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Lofepramine hydrochloride 10µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram (Wiggins *et al.,* 1991).

Preparation of Standard Stock Solution

10mg of Lofepramine hydrochloride was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of Working Standard Solution

From stock solutions of Lofepramine hydrochloride 1.0 ml was taken and diluted up to 10 ml from this solution 0.5, 1.0, 1.5, 2.0, 2.5ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Analysis of tablets formulation

Take 20 tablets and determine the average weight, weight equivalent to 10mg of Lofepramine hydrochloride was transferred to 10ml volumetric flask and dissolved in mobile phase. The solution was shaking vigorously for 20mins and filtered through Whattman filter paper no. 41, then volume was made up to mark with mobile phase. From the above solution 1ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100µg/ml. From the above solution 1ml of solution was taken and diluted to 10ml with mobile phase to get a solution containing 10µg/ml of Lofepramine hydrochloride. The amounts of Lofepramine hydrochloride in tablet formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation (Foda *et al.,* 1988)

Validation

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 2 to 10µg/ml) concentrations and areas

for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug was plotted. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration (Bhardwaj *et al.,* 2015).

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

Intermediate Precision

Day to Day

The statistical analysis method was carried out and the data is presented in table.

Analyst to Analyst

The intermediate precision expresses with in laboratories variation (different days, different analysts, different equipment etc). The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of Lofepramine hydrochloride was studied.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation

50 mg of drug sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Alkaline hydrolysis

50 mg of the drug sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Oxidative degradation

50 mg of the drug sample was taken into a 50 ml round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring

for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Thermal degradation

50 mg of the drug sample was taken in to a petridish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

RESULTS AND DISCUSSION

The development and validation of the stability-indicating HPLC method for the estimation of Lofepramine hydrochloride have demonstrated significant results, ensuring that this method is suitable for quality control and stability testing in pharmaceutical formulations. The detailed analysis of various system suitability parameters, linearity, precision, recovery studies, and robustness provides a solid foundation for the reliability of this method in both routine testing and stability assessments.

The chromatographic conditions outlined in Table 1, which utilize a 50:50 mixture of methanol and acetonitrile as the mobile phase, were chosen for their ability to provide well-resolved peaks for Lofepramine hydrochloride. The column dimension (250 mm x 4.60 mm) and particle size (5 µm) ensured efficient separation, while the room temperature and flow rate of 1.0 mL/min contributed to stable and reproducible results. The retention time of 6.115 ± 0.3 minutes, as reported, indicates that the method is consistent and can be reliably used for Lofepramine hydrochloride estimation in tablet formulations.

Table 2 demonstrates the system suitability for Lofepramine hydrochloride. The results from multiple replicates show that the retention time (6.115 ± 0.0017 min) was consistent, and the low standard deviation (S.D. = 0.0017) indicates that the system is stable and reliable. The area under the curve (AUC) and theoretical plates were also consistent across replicates, confirming the method's resolution and precision. The tailing factor (1.187) is within the acceptable range (1.0-2.0), indicating that the peaks are symmetrical and that the method provides good separation without distortion.

The results from the linearity study (Table 3) confirm that the method exhibits excellent linearity within the range of 2–10 µg/mL for Lofepramine hydrochloride. The calculated relative standard deviations (% RSD) for each concentration were very low (ranging from 0.135% to 0.456%), which indicates the high precision of the method across a broad concentration range. The good linearity (R^2 value) of the calibration curve assures that the method is appropriate for accurate quantification of the drug in tablet formulations.

Precision was evaluated both intra-day and inter-day, as shown in Tables 8 and 9, with results demonstrating minimal variation in the percentage of label claim for Lofepramine hydrochloride. The intra-day % RSD values were 0.627%, while the inter-day % RSD values were 0.475%, both of which are well within the acceptable limits for pharmaceutical quality control (≤2%). These low values indicate that the method is highly precise and reproducible over time.

Recovery studies (Table 5) further validate the accuracy of the method. The % recoveries of Lofepramine hydrochloride at 80%, 100%, and 120% of the standard concentration were close to 100%, with values ranging from 96.25% to 99.75%. These results indicate that the method is accurate and capable of recovering Lofepramine hydrochloride without interference from formulation excipients or other matrix effects.

The robustness study, as presented in Table 10, assessed the method's resilience to minor variations in temperature, flow rate, and mobile phase ratio. The % RSD values remained low for changes in temperature (1.1% to 1.25%), flow rate (0.48% to 0.89%), and mobile phase ratio (0.89% to 1.05%), demonstrating that the method is robust and reliable under slightly altered conditions. This robustness is critical for routine quality control, where small variations in instrument settings are common.

Forced degradation studies (Table 11) revealed important information about the stability of Lofepramine hydrochloride under various stress conditions. The drug was found to be highly stable under standard conditions (99.95% recovered), but degradation occurred under acidic (7.77% decomposition), alkaline (5.44% decomposition), oxidative (14.44% decomposition), and photolytic (16.68% decomposition) conditions. This highlights the need for stability-indicating methods to ensure that any degradation products do not affect the drug's safety and efficacy. The method developed successfully detected these degradation products, making it a suitable tool for stability testing.

The method was also applied to the analysis of Lofepramine hydrochloride in tablet formulations (Table 4). The percentage of Lofepramine hydrochloride found in the formulation was very close to the label claim, with the mean recovery rate being 98.3%. The low % RSD (1.539%) further supports the method's accuracy and precision in real-world pharmaceutical applications. Additionally, the tablet analysis demonstrated that the method is applicable for routine quality control in the pharmaceutical industry.

Table 1: Selection of separation variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Methanol	50
ACN	50
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 µl
Detection wavelength	294 nm
Retention time Lofepramine hydrochloride	6.115 $\pm$ 0.3 min

Table 2: Result of system suitability parameters for Lofepramine hydrochloride

System suitability Parameter →	RT	AUC	Theoretical plates	Tailing factor
Rep-1	6.115	4598.856	2895	1.15
Rep-2	6.118	4582.336	2896	1.25
Rep-3	6.115	4575.325	2878	1.16
Mean	6.116	4585.506	2889.667	1.187
S.D.	0.0017	4598.856	10.116	0.055

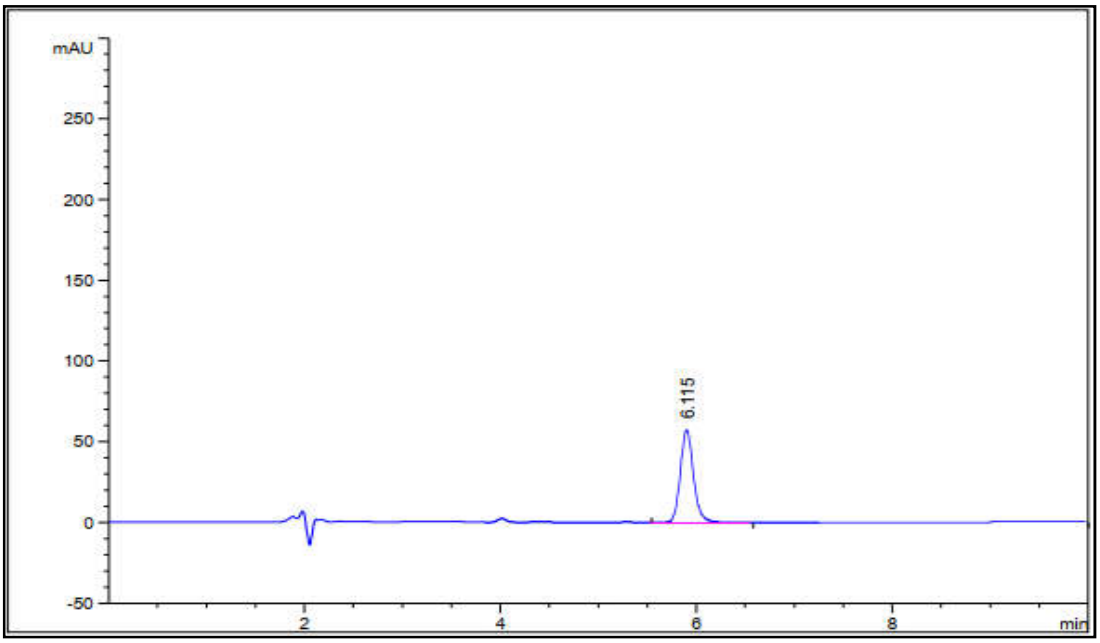

Figure 1: Chromatogram of Standard

Table 3: Result of Linearity of Lofepramine hydrochloride

Conc. (µg/ml)	2	4	6	8	10
Rep.	0	0	0	0	0
1	2356.589	4598.856	6898.856	9285.658	11589.669
2	2365.589	4582.336	6875.365	9265.587	11685.658
3	2347.854	4575.325	6866.885	9288.658	11598.856
Mean	2356.677	4585.506	6880.369	9279.968	11624.728
S.D.	8.868	12.081	16.562	12.544	52.967
R.S.D%	0.376	0.263	0.241	0.135	0.456

Table 4: Result of analysis for Lofepramine hydrochloride in tablets formulation

Std Conc. µg/ml	Lofepramine hydrochloride
	10 (µg/ml)
Rep-1	9.66
Rep-2	9.88
Rep-3	9.95
% found *	
Rep-1	96.6
Rep-2	98.8
Rep-3	99.5
Mean	98.3
SD	1.513
% RSD	1.539

*Each reading is mean reading of three batch of formulation

Table 5: Recovery Studies of Formulation

Level of Recovery (%)	80%	100%	120%
Amount present (mg)	5 5 5	5 5 5	5 5 5
Amount of Std. added (mg)	4 4 4	5 5 5	6 6 6
Amount recovered (mg)	3.98 3.99 3.85	4.95 4.88 4.75	5.88 5.98 5.88
% Recovery	99.50 99.75 96.25	99.00 97.60 95.00	98.00 99.67 98.00

Table 6: Statistical Validation of Recovery Studies

Level of Recovery (%)	% Recovery	Standard Deviation*	% RSD
80	98.50	97.20	98.56
100	1.953	2.030	0.962
120	1.982	2.088	0.976

Table 7: Results of analysis Data of Tablets Formulation

Drug	Label claim (%)	Amount found* (%)	Label claim (%)	S.D.	% RSD
Lofepramine hydrochloride	70	69.50	99.28	0.115	0.332

Table 8: Intra-day and Inter-day Precision

Intra-day Precision		Inter-day Precision	
	% Label Claim		% Label Claim
	Lofepramine hydrochloride		Lofepramine hydrochloride
After 1hr	99.85	First day	98.45
After 2hr	99.32	Second day	97.95
After 3hr	98.45	Third day	97.52
After 4hr	98.45		
After 5hr	98.65		
After 6hr	98.42		
Mean	98.857	Mean	97.973
SD	0.620	SD	0.465
% RSD	0.627	% RSD	0.475

Table 9: Analyst to Analyst

Analyst	Label claim	Amount found*	Label claim (%)	S.D.	% RSD
1	70	69.45	99.21	0.125	0.1656

Table 10: Result of Robustness of Formulation

Compound	% RSD in Normal	Changed Condition n= 6	
Temperature		- 5 ^{o}C	+ 5 ^{o}C
Lofepramine hydrochloride	0.89	1.1	1.25
Flow rate		(-10%)	(+10%)
Lofepramine hydrochloride	0.48	0.55	0.89
Mobile phase ratio		- 2 %	+ 2 %
Lofepramine hydrochloride	0.89	0.95	1.05

Table 11: Results of forced degradation studies of Lofepramine hydrochloride

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.95	0
Acidic hydrolysis	92.23	7.77
Alkaline hydrolysis	94.56	5.44
Oxidative degradation	85.56	14.44
Photolytic degradation	83.32	16.68

CONCLUSION

In conclusion, the stability-indicating HPLC method developed for the estimation of Lofepramine hydrochloride meets all the essential criteria for accuracy, precision, and robustness. The method is suitable for the routine analysis and stability testing of Lofepramine hydrochloride in tablet formulations. It effectively separates the active pharmaceutical ingredient from its degradation products, providing reliable data for quality control and stability studies. Furthermore, the validation studies demonstrate that the method complies with international standards for analytical methods, ensuring its reliability and consistency in pharmaceutical practice.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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