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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF LIDAMIDINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
A rapid and precise reverse phase High Performance Liquid Chromatographic method has been developed for the validated of Lidamidine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Luna C18 (4.6×150mm, 5µm) column using a mixture of Methanol: Water (50:50%v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 270nm. The retention time of the Lidamidine was 2.826 ±0.02min. The method produce linear responses in the concentration range of 20-100ppm of Lidamidine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Lidamidine, RP-HPLC, validation.

INTRODUCTION
Lidamidine is an investigational drug that has antidiarrheal properties. The mechanism of action remains controversial, but animal studies have shown it to be free of narcotic and classic anticholinergic activity. The drug is thought to act as an α-adrenergic agonist through its effect on α_2-receptors. In animal models, lidamidine has effectively inhibited diarrhea induced by castor oil, prostaglandin E_2, carbachol, serotonin, and bile acid. Literature review of Lidamidine shown there were several method like Biotransformation[(1)] of Lidamidine in rat and analytical methods like GLC[(2)] and only few methods were reported for RP-HPLC for the estimation of this drug in bulk and in its formulation. Hence the present work targeted to develop a new precise, accurate and sensitive RP-HPLC[(3-7)]method for the determination of Lidamidine in API and formulation. The developed method validated as per ICH guidelines.[8-10]

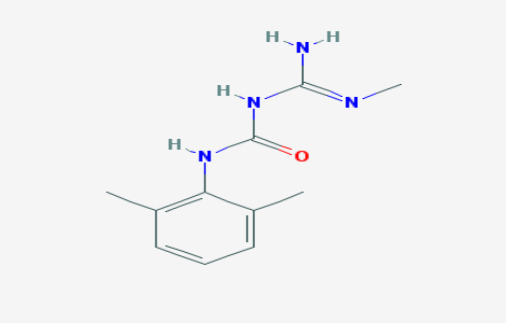

Figure 1: structure of Lidamidine.

MATERIALS AND METHODS
Chemicals and reagents used
Lidamidine as pure standard reference drug was obtained from Sura labs, Hyderabad, India. Acetonotrile, Methanol and water used were of HPLC grade and purchased from MERCK specialties Private Limited, Mumbai, India.

Apparatus
HPLC analysis was performed on chromatographic system of water 2695 separation module with empower software liquid chromatography comprising water 996 photo diode array detector, Symmetry C18 5µm (4.6×150mm, 5 µ) was used and an equipped with auto sampler. Derivative spectral and photometric absorbance measurements are done on UV spectrophotometer with software UV win, Lab India make 3092. 10mm path length quartz cells were used.

Experimental conditions
Chromatographic separation achieved using an analytical Symmetry C18 5µm (4.6×150mm, 5 µ). Mobile phase consisted of Methanol: Water (50:50 v/v). The elution was achieved isocratically at a flow rate of 0.9ml/min with injection volume of 10µl, column temperature was maintained at 40ºC and chromatograph was recorded at wavelength 270nm

Preparation of standard solution
Accurately weigh and transfer 10 mg of Lidamidine 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 0.6ml of the above Lidamidine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Mobile Phase Optimization:
Initially the mobile phase tried was Acetonitrile: Water and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Water in proportion 50:50 v/v respectively.

Optimization of Column
The method was performed with various columns like C18 column, X- bridge column, Xterra, and C18 column. Symmetry C18 5µm (4.6×150mm) 5 µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

PREPARATION OF MOBILE PHASE
Preparation of mobile phase
Accurately measured 500 ml (50%) of HPLC Water and 500 ml of Methanol (50%) were mixed and degassed by sonication for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the diluent.

Method development
Some important parameters like pH of the mobile phase, concentration of the acid, were tested for a good chromatographic separation. Trials showed that mobile phase with reverse phase C18 column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Lidamidine as carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Lidamidine was recorded in Figure 2. Now the sample solution was injected separately and chromatogram was recorded until the reproducibility of the peak areas were satisfactory (Figure 3).

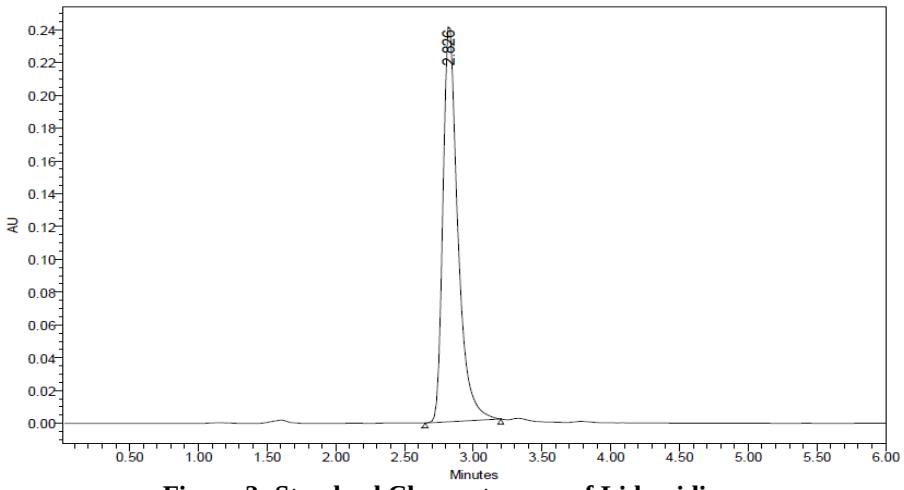

Figure 2: Standard Chromatogram of Lidamidine.

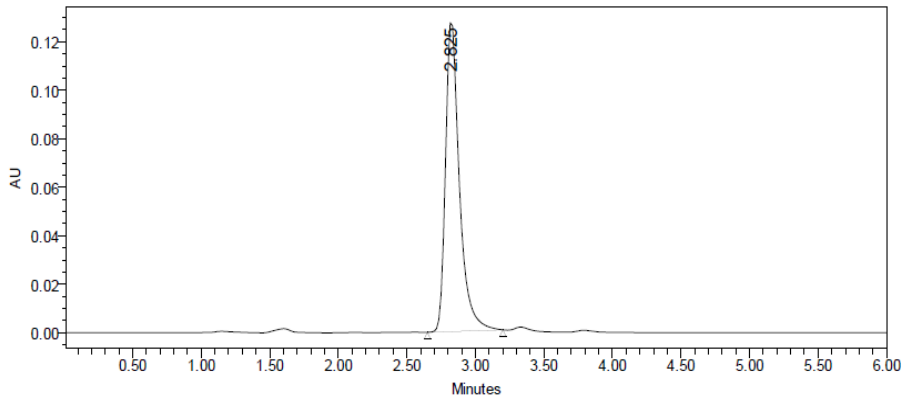

Figure 3: Sample Chromatogram of Lidamidine.

Analytical method validation
HPLC method was validated according to the International Conference on Harmonization guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as linearity, precision, accuracy, system suitability limit of detection, limit of quantification and robustness.

Linearity
Inject each level (20, 40, 60, 80 and 100µg/mL) solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area in the concentration range of 20 – 100 µg/mL. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients.

PRECISION
Repeatability
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was calculated.

Intermediate precision
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions. For intermediate precision % RSD was calculated from repeated studies.

Accuracy
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Lidamidine and calculate the individual recovery and mean recovery values.

Robustness
Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for injections peak area values of each change in condition.

System suitability
This parameter used to know whether the HPLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting five standard solutions of Lidamidine and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

Specificity
Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected.

Limit of detection
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Quantitation limit
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10 \times \sigma / S$$

RESULTS AND DISCUSSION
Linearity and range
Linearity and range estimated by constructing the calibration curve by taking concentration on X-axis and peak area on Y-axis of solutions (20, 40, 60, 80 and 100µg/mL) prepared from standard stock solution) and calculate the correlation coefficient. Correlation Coefficient (r) is 0.99, and the intercept 21287. These values meet the validation criteria as shown in Figure 4 and linearity values tabulated in Table 1.

Table 1:Chromatographic data for linearity study.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	20	282417
66	40	526995
100	60	783937
166	80	1031164
133	100	1237297

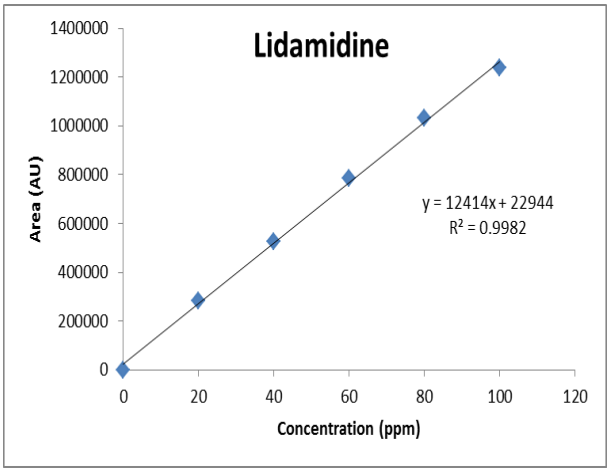

Figure 4: Calibration curve of lidamidine.

PRECISION
Intermediate precision
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The results were shown in Table2 for analyst 1and Table-3 for analyst 2. Calculated % RSD values were less than 2.

Table 2: Results of Intermediate precision for analyst 1 for lidamidine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Lidamidine	2.823	895311	125747	4510	1.4
2	Lidamidine	2.827	896783	122578	4002	1.4
3	Lidamidine	2.828	895237	124365	4235	1.4
4	Lidamidine	2.828	894206	124057	4235	1.4
5	Lidamidine	2.825	895085	125410	4015	1.4
6	Lidamidine	2.822	896041	129241	3998	1.3
Mean			895443.8			
Std. Dev.			879.931			
% RSD			0.09			

Table 3: Results of Intermediate precision Analyst 2 for Lidamidine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Lidamidine	2.833	893627	132891	4986	1.4
2	Lidamidine	2.836	892682	130515	5123	1.4
3	Lidamidine	2.827	896754	129139	4081	1.4
4	Lidamidine	2.827	896754	129139	4150	1.4
5	Lidamidine	2.823	895311	125747	4051	1.3
6	Lidamidine	2.827	896783	122578	4150	1.4
Mean			895318.5			
Std. Dev.			1793.234			
% RSD			0.2			

Repeatability

Multiple sampling from a sample solution was done and five working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas Standard Deviation and % Relative Standard Deviation are mentioned in Table 4.

Table 4: Results of repeatability for Lidamidine.

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Lidamidine	2.824	894562	128135	3981	1.3
2	Lidamidine	2.827	896754	129139	4213	1.4
3	Lidamidine	2.833	893627	132891	4562	1.4
4	Lidamidine	2.833	893750	129914	4562	1.4
5	Lidamidine	2.836	892682	130515	4610	1.4
Mean			894275			
Std.dev			1537.936			
%RSD			0.171976			

Accuracy

Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Lidamidine and calculate the individual recovery and mean recovery values. The accuracy results for Lidamidine are recorded in Table 5.

Table 5: The accuracy results for Lidamidine.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	406872	30	29.8	99.3	99.5%
100%	766853.3	60	59.9	99.8	
150%	1143813	90	89.6	99.5	

Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.0ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Lidamidine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Lidamidine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results were recorded in Table 6.

Table 6: Results for Robustness.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	1817227	2.826	4531	1.4
Less Flow rate of 0.8mL/min	1005760	3.13	4921.2	1.4
More Flow rate of 1.0mL/min	819776	2.589	4493.3	1.4
More Organic phase	922032	2.514	3834.7	1.3
Less Organic phase	893128	3.344	5032.7	1.3

System suitability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were cited in table 7.

Table 7: Results of system suitability for Lidamidine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Lidamidine	2.824	1819564	249911	3965	1.4
2	Lidamidine	2.825	1822439	252600	3998	1.4
3	Lidamidine	2.827	1819738	255482	4015	1.4
4	Lidamidine	2.822	1816041	249241	3975	1.4
5	Lidamidine	2.830	1812710	245336	4215	1.4
Mean			1818098			
Std. Dev.			3773.09			
% RSD			0.2			

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Lidamidine in drug product. The results for specificity of Lidamidine were cited in Table 8 and Table 9.

Table 8: Peak results for assay standard.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Lidamidine	2.828	892717	124236	1.4	3922.9	1
2	Lidamidine	2.829	899298	124029	1.4	3883.2	2
3	Lidamidine	2.828	891366	125525	1.4	4023.9	3

Table 9: Peak results for Assay sample.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Lidamidine	2.826	896838	124869	1.4	3928.6	1
2	Lidamidine	2.825	898292	128687	1.4	4568.8	2
3	Lidamidine	2.833	901496	129200	1.4	5693.0	3

Limit of detection for lidamidine

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The value was found to be 4.4µg/ml.

Quantitation limit of lidamidine

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. The value was found to be 13.3µg/ml.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Lidamidine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Lidamidine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Lidamidine in bulk drug and in Pharmaceutical dosage forms.

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