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Development and validation of RP-HPLC Assay method for determination of lacidipine in tablets

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Abstract:

A simple, sensitive and accurate isocratic reverse phase high performance liquid chromatography method was developed for determination of lacidipine in tablets. The effective separation was achieved on Thermo Hypersil BDS C18 75 x 3.5 mm column with particle size 3.0µm. The mixture of buffer and methanol in the ratio 30: 70 v/v used as a mobile phase. The buffer was prepared as 4.0 g of ammonium acetate in 2000 ml of purified water and adjusts the pH 6.5 with acetic acid. The flow rate of the mobile phase was 1.2 mL/min and the total elution time was 5 minutes. The UV detection wavelength was carried at 242 nm and experiments were conducted at 40°C. The developed method was validated in terms of system suitability, selectivity, linearity, precision, accuracy and robustness for quantification of lacidipine following the ICH guidelines.

Key Words: Lacidipine tablets, Method development, Hypersil BDS C18 and RP-HPLC

1. INTRODUCTION:

Lacidipine [1] is a calcium channel blocker drug. Chemically Lacidipine is (E)-4-[2-[3-(1,1-Dimethylethoxy)-3-oxo-1- propenyl]phenyl]-1,4-dihydro-2,6-dimethyl-3,5pyridine dicarboxylic acid diethyl ester (Figure 1). It has a molecular formula of $C_{26}H_{33}NO_6$ and a molecular weight of 455.55 g/mol. Literature survey reveals that several analytical methods have been reported for the estimation of lacidipine by LC-DAD [2], High Performance Thin Layer Chromatography [3], LC-MS [4,5] and UV [6] method. Some HPLC [7-9] methods were reported in the literature for the determination of lacidipine. The aim of this study was to develop a RP-HPLC method, which could be employed for the routine analysis of the drug in pharmaceutical dosage forms using simple mobile phase composition. The present work describes a simple, isocratic RP-HPLC method for the determination of lacidipine tablets as per ICH guidelines [10-12].

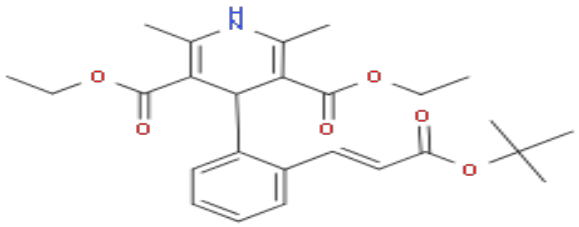

Fig: 1 Chemical structure of lacidipine

2. EXPERIMENTAL: MATERIALS AND REAGENTS:

2.1 Instrumentation and software:

A high performance liquid chromatography system manufactured by Waters which consist of PDA detector, Quaternary solvent manager, sample manager, column heating compartment was used for assay determination of lacidipine in tablets. HPLC instrument was controlled by empower software. A Thermo Hypersil BDS C18 75 x 3.5 mm, column with particle size of 3.0µm was used as stationary phase for chromatographic separation. Sartorius semi micro analytical balance was used for all weighing, Thermo pH meter was used for buffer pH adjustment, and sonicator used to dissolve the solutions.

2.2 Buffer Preparation:

The buffer was prepared as 4.0g of ammonium acetate in 2000 ml of purified water and adjusts the pH 6.5 with acetic acid.

2.3 Preparation of mobile phase:

Mixture of buffer solution and methanol in the ratio of 30:70v/v was used as mobile phase.

2.5 Preparation of standard solutions:

Weighed accurately and transferred 25 mg of lacidipine standard into a 100 mL volumetric flask add about 35 mL of methanol, sonicate to dissolve the material completely, dilute to volume with water and mix well.

2.6 Preparation of sample solutions:

Weighed accurately and transferred lacidipine tablet powder equivalent to 25 mg of lacidipine into a 100 mL volumetric flask, add 35mL of methanol, sonicate for 10 minutes with intermittent shaking, dilute to volume with water and mix well.

3. Method validation parameters

The system suitability was conducted using standard preparation and evaluated by injecting six replicate injections. Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting Blank into the chromatographic system and evaluated by show any peak at the retention time of analyte. Linearity is performed in the range of 50 to 150% of specification

limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also precision is performed at 100% level by injecting six times into the chromatographic system.

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted using lacidipine and evaluated by making six replicate injections. The Accuracy of the method by recoveries of lacidipine sample solutions at different concentration levels ranging. The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

4. RESULTS AND DISCUSSION:

4.1 Optimization of chromatographic conditions:

The following analytical parameters having major role during the method development and validation includes selection of appropriate chromatographic conditions/factors like detection wavelength, selection of stationary and mobile phases, column temperature and injection volume. The wavelength of 242 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify lacidipine in tablets. Preliminary development trials were performed with various columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Hypersil BDS C18 75 x 3.5 mm, 3.0µm column there a significant improvement in the peak shapes with 0.8 tailing factor.

5. Method validation:

5.1 System suitability:

The RSD from six replicate injections of lacidipine standard preparation was 0.4 %. System suitability data is given in Table-1.

Table-1: System suitability results of lacidipine

Injection No.:	Lacidipine Peak Area
1	2816070
2	2817747
3	2811955
4	2843678
5	2824271
6	2823178
Mean	2822817
%RSD (NMT 2.0%)	0.4
Tailing factor (NMT 1.5)	0.8

5.2 Selectivity:

Performed the specificity parameter of the method by injecting diluent, standard preparation sample preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved no peak observed at retention time of lacidipine. Specificity results of lacidipine given in the Table-2. The typical selectivity chromatograms are shown in Figure-2, 3 & 4.

2. Retention time of lacidipine in Sample and Standard

S.No.	Compound	Retention time
1	Blank (Diluent)	--
2	Standard	3.566
3	Sample solution	3.581

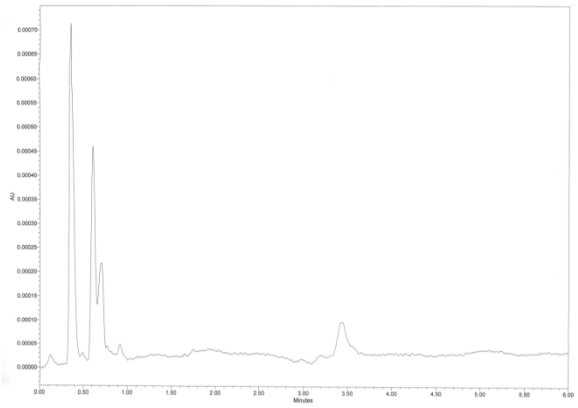

Fig-2: Blank chromatogram

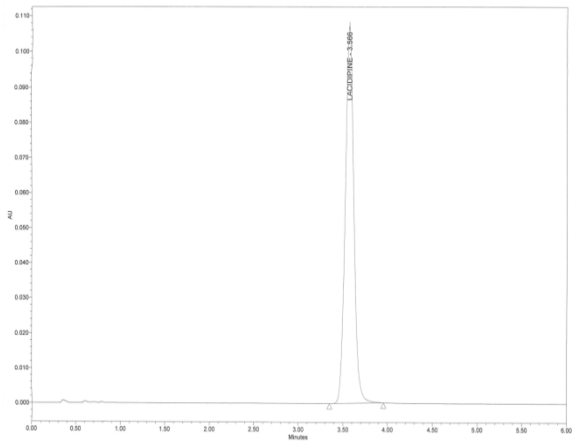

Fig-3: Lacidipine standard chromatogram

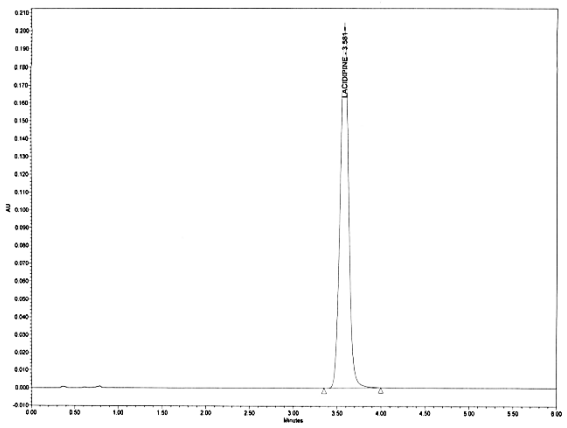

Fig-4: Lacidipine sample chromatogram

5.3 Linearity:

To demonstrate the linearity with lacidipine in the range from 50% to 150% of specification limit**.** Correlation coefficient of lacidipine was 0.998.The linearity results shown in the below Table -3. Linearity curves of lacidipine and its impurities shown in the Figure-5.

Table 3: Linearity results

S.No.	Lacidipine Concentration µg/ml	Concentration Level (%)	Peak Area
01	15	50	1321450
02	24	80	2100015
03	30	100	2715620
04	38	125	3284881
05	46	150	4165845
Correlation coefficient		0.998	

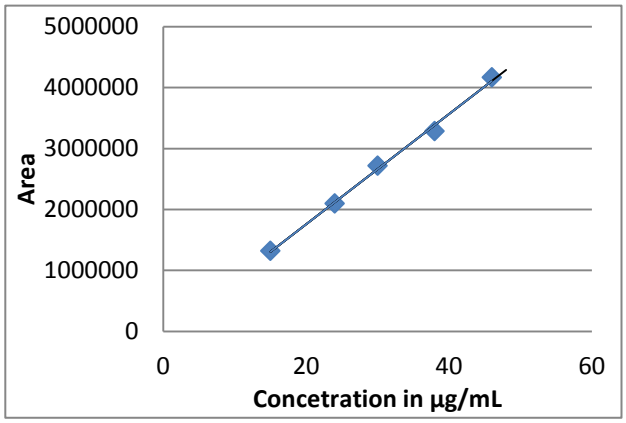

Figure-5 Linearity curves of lacidipine

5.4 Precision:

The precision of test method was validated by relative standard deviation of individual % LC of lacidipine from the six preparations. The precision results are given Table-4.

Table-4 Precision results

Injection	Peak Area	%LC
1	2785615	99.8
2	2798995	100.1
3	2774622	100.0
4	2791220	101.1
5	2798458	101.3
6	2788621	99.9
Mean		100.4
%RSD (NMT: 3.0%)		0.6

5.5 Accuracy:

The accuracy study was performed at three levels 50%, 80%, 100%, 125% & 150% of sample concentration. The percentage recovery of lacidipine in tablets ranged from 99.2 to 100.3 respectively.

Table 5: Accuracy results

Sample	% Recovery	Mean
50%	99.8	99.7
	99.1	
	100.1	
80%	99.6	99.5
	99.8	
	99.2	
100%	99.8	100.0
	100.2	
	100.1	
125%	100.1	100.1
	99.9	
	100.3	
150%	99.7	99.7
	99.8	
	99.5	

5.6 Robustness

The method robustness was studied by injecting the standard solution at change in the pH of buffer solution, percentage of organic modifier (methanol) and column temperature. The results were obtained as shown in the below Table-6.

Table-6: Robustness results

Condition	Tailing factor (NMT: 1.5)	% RSD (NMT: 2.0)
Normal Condition (as such condition)	0.8	0.4
Buffer pH 6.3	1.0	0.5
Buffer PH 6.7	1.0	0.4
Column temperature 35°C	0.9	0.4
Column temperature 45°C	1.0	0.4
Change in organic component - 10%	1.0	0.5
Change in organic component + 10%	1.0	0.3

6. CONCLUSION:

A simple, isocratic HPLC method has been developed and validated for the determination of Lacidipine in tablets. The developed method has been found to selective, sensitive, precise, and robust. The method can be directly adopted in quality control laboratories for routine analysis with respect to determination and quantification of lacidipine and also for the analysis of stability samples.

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