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Research Article

A Novel RP HPLC Method for Development and Validation of Cilnidipine In Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, Precise, accurate RP-HPLC method was developed and validated for the estimation of cilnidipine in pharmaceutical dosage forms. separation was achieved on Symmetry C18 column (4.6 x 150mm), with mobile phase consisting of ortho phosphoric acid buffer pH 4 and Acetonitrile in 60:40, V/V. The flow rate was maintained at 1 ml/min and the analyte was monitored at 240nm wavelength. The retention time for cilnidipine was found to be 2.35 min. The linearity of the method was observed in the concentration range of 5-25ppm and correlation coefficient was found to be 0.999. The percentage assay of Cilnidipine was found to be 98.733%.

The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in flow rate and analysis being performed on different days.

The method was validated for its accuracy, precision and system suitability. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the routine estimation of Cilnidipine in pharmaceutical dosage forms.

Key-words: Cilnidipine, RP- HPLC, Validation etc.

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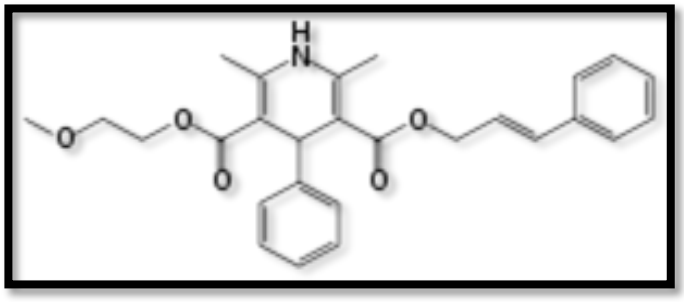
INTRODUCTION: -

High-pressure liquid chromatography[1] is used in analytical development to quantitate the active pharmaceutical ingredient (API) and to evaluate impurity and degradation product profiles of drug substances (DS) and drug products (DP).

Cilnidipine (Fig. 1) is chemically *O*3-(2-methoxyethyl) *O*5-[(*E*)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine- 3, 5 dicarboxylate. Cilnidipine is an oral Anti-Hypertensive drug approved for use in patients with Hypertension.

Fig. 1: Chemical structure of Cilnidipine



The literature review reveals that there are some analytical methods reported for Cilnidipine by RP-HPLC[2] method and most of the work done on biological fluids. Various analytical methods like UV[3], HPTLC[4], LC-MS[5] are reported for the analysis of this compound individually. The developed method will be validated in terms of accuracy, precision, linearity, robustness[6] and ruggedness, and results will be validated statistically according to ICH guidelines[7].

MATERIALS AND METHODS: -

Chemicals/ Reagents and Solvents:

Table 1: Chemicals/ Reagents

S. No.	Chemicals/standards and reagents	Grade	Manufacturer
1	Potassium dihydrogen phosphate	HPLC	Fisher
2	Ortho phosphoric acid	HPLC	Fisher
3	HPLC Grade Methanol	HPLC	Merck
4	HPLC Grade Acetonitrile	HPLC	Merck
5	Double Distilled Water	HPLC	Merck
6	Cilnidipine	Gift Sample	Pharm Tech, Hyderabad

Instruments and Equipment:

Table 2 : Instruments and Equipment

S.NO.	Instruments and Equipment's	Software	Model	Manufacturer
1	HPLC	N2000 chromatographic system	Waters 515pump, Detector 2487	WATERS
2	UV-Spectrophotometer	UV Analyst	UV- 2310	TECHCOMP
3	Weighing Balance	N/A	XEX 200	SHIMADZU
4	Sonicator	N/A	SE60US	ENERTECH
5	pH Meter	N/A	AD102U	ADWA

ANALYTICAL METHOD DEVELOPMENT

Optimization of UV conditions:

Initially method development work was started by taking UV-visible spectra from 400-200 nm of Cilnidipine (10 ppm), standard solution. By observing the spectra of standard solutions λmax 240 nm was taken for trials to develop UV method.

The objective of this experiment was to optimize the assay method for estimation of Cilnidipine based on the literature survey made, few of the trails mentioned which describes how the optimization was done.

TRIALS FOR CILNIDIPINE -

Trail: 1Chromatographic conditions:

Mobile phase : Buffer pH 6: Methanol: (60: 40)
Flow rate : 0.8 ml/min
Column : Symmetry C_{18} (4.6 x 100 mm, 3.5 µm)
Detector wavelength : 240 nm
Column temp : Ambient
Injection volume: 20 µl

Trail: 2Chromatographic conditions:

Mobile phase : Buffer pH 4: Methanol (35:65)
Flow rate : 1 ml/min
Column : Symmetry C_{18} (4.6 x 100 mm, 3.5 µm)
Detector wavelength : 240 nm
Column temp : Ambient
Injection volume : 20 µl

Trail: 3Chromatographic conditions:

Mobile phase : Buffer pH 4: Methanol: Acetonitrile (30:50:20)
Flow rate : 0.7 ml/min
Column : Symmetry C_{18} (4.6 x 100 mm, 3.5 µm)
Detector wavelength : 240 nm
Column temp : Ambient
Injection volume : 20 µl

Table 3: Shows Optimized Method Parameters

PARAMETERS	CONDITIONS
Column (Stationary Phase)	Symmetry C_{18} (4.6 x 100 mm, 5µm) or equivalent
Mobile Phase	Ortho phosphoric acid buffer (pH 4.0): Acetonitrile in ratio of 60: 40 %
Flow rate	1(ml/min)
Run time	5 (min)
Column temperature	Ambient
Volume of injection loop	20 (µl)
Detection wavelength	240 (nm)
Retention time	2.35 min

Preparation of the Cilnidipine Standard & Sample Solution:

Preparation of Standard Solution:

Accurately weighed and transferred 10 mg of Cilnidipine working standard into a 10 ml clean dry volumetric flask and added about 7 ml of diluent and sonicated to dissolve it completely and made the volume up to the mark with the same solvent.

Further pipetted out 0.3 ml of Cilnidipine of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent

Sample Solution Preparation :(Assay)

Accurately weighed and transferred 10 mg of Cilnidipine tablet powder into a 10 ml clean dry volumetric flask, added about 7 ml of Diluent and sonicated to dissolve it completely and made the volume up to the mark with the same solvent.

Further pipetted out 0.3 ml of Cilnidipine of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent.

FORMULA:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt.}}{\text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation.
AS= average area counts of standard preparation.
WS = Weight of working standard taken in mg.
WT = Weight of working sample taken in mg
DS = Dilution of standard
DT = Dilution of test sample
P = Percentage purity of working standard
LC = Label Claim of drug, mg/ml.

ANALYTICAL METHOD VALIDATION

Validation:

Establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce a product meeting its predetermined specification and quality attributes.

(a) Accuracy
(b) Precision
(c) Linearity
(e) Limit of detection
(f) Limit of quantitation
(g) Robustness
(h) System Suitability

Accuracy:

The accuracy of the method was assessed by recovery study of Cilnidipine in the dosage form at three concentration levels. A fixed amount of pre analysed sample was taken and standard drug was added at 50%, 100% and 150% levels. Each level was repeated three times. Individual recovery and mean recovery values were calculated and Cilnidipine results were reported.

Preparation of Standard stock solution:

Accurately weighed and transferred 10 mg Cilnidipine working Sample into a 10 ml clean dry volumetric flask and added about 7ml of Diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further pipetted out 0.3 ml of Cilnidipine of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weighed and transferred 5 mg of Cilnidipine working standard into a 10 ml clean dry volumetric flask added about 7 ml of diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further pipetted out 0.3 ml of Cilnidipine of the above stock solution and 0.3 ml Working Sample solution into a 10 ml volumetric flask and diluted up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Pipetted out 0.6 ml of Cilnidipine of the above stock solution and 0.3 ml Working Sample solution into a 10 ml volumetric flask and diluted up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

Pipetted out 0.6 ml of Cilnidipine of the above stock solution and 0.3 ml Working Sample solution into a 10 ml volumetric flask and diluted up to the mark with diluent.

Acceptance Criteria: The % Recovery for each level should be between 98.0% to 102.0%

Precision:

Preparation of stock solution:

Accurately weighed and transferred 10 mg of Cilnidipine working standard into a 10 ml clean dry volumetric flask and added about 7 ml of diluent and sonicate to dissolve it completely and made volume up to the mark with the same solvent. Further pipetted out 0.3 ml of Cilnidipine of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent.
The % RSD for the area of five replicate injections was found to be within the specified limits . Results were reported.
Acceptance Criteria:
The % RSD for the area of five sample injections results should not be more than 2 %.

Intermediate Precision/Ruggedness:
Preparation of stock solution:
Accurately weighed and transferred 10 mg of Cilnidipine working standard into a 10 ml clean dry volumetric flask and added about 7 ml of diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further pipetted out 0.3 ml of Cilnidipine of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. The % RSD for the area of five replicate injections was found to be within the specified limits. Results were reported in Table number 10.
Preparation of Level – IV (20 ppm of Cilnidipine**):**
0.4 ml of stock solution was taken in 10 ml of volumetric flask and diluted up to the mark with diluent.
Preparation of Level – V (25 ppm of Cilnidipine**)**
0.5 ml of stock solution was taken in 10 ml of volumetric flask and diluted up to the mark with diluent.
A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and correlation coefficient was calculated.
The Cilnidipine results were reported in Table number 11.
Acceptance Criteria: Correlation coefficient should be not less than 0.999.

Limit of Detection:
The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

Limit of Quantification
The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

Robustness:
As part of the Robustness, deliberate change in the flow rate, mobile phase composition, were made to evaluate the impact on the method.

Preparation of stock solution:
Standard solution 30 ppm of Cilnidipine was prepared and analysed using the varied flow rates along with method flow rate.

Effect of Variation of Mobile Phase Ratio:
The organic composition in the Mobile phase was varied from 40% to 60%. Standard solution 30 µg/ml of Cilnidipine was prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method. The results were reported in Table number 13.

System Suitability Parameters:
System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor, retention time and theoretical plates. System suitability parameter Results were reported in Table number 14.

Table 4 Criteria for Validation of the Method

CHARACTERISTICS	ACCEPTABLE RANGE
Accuracy	Recovery (98-102%)

Precision	RSD < 2%
Intermediate precision	RSD < 2%
LOD	S/N > 2 or 3
LOQ	S/N > 10
Linearity	Correlation Coefficient(r)>0.99
Range	80-120%
Stability	>24h or >12h

RESULTS AND DISCUSSION:-

The present investigation was to develop a new method and validation of cilnidipine in pharmaceutical dosage form by RP-HPLC.

UV SPECTRUM OF CILNIDIPINE

UV spectrum was recorded for cilnidipine individually and ƛ max of cilnidipine was found to be 240 nm.

TRIALS:

Standard solution of cilnidipine was determined under developed chromatographic conditions (trails) indicating satisfactory retention time and area. From the experiment it was found that cilnidipine can effectively be analysed by using the RP- HPLC method.

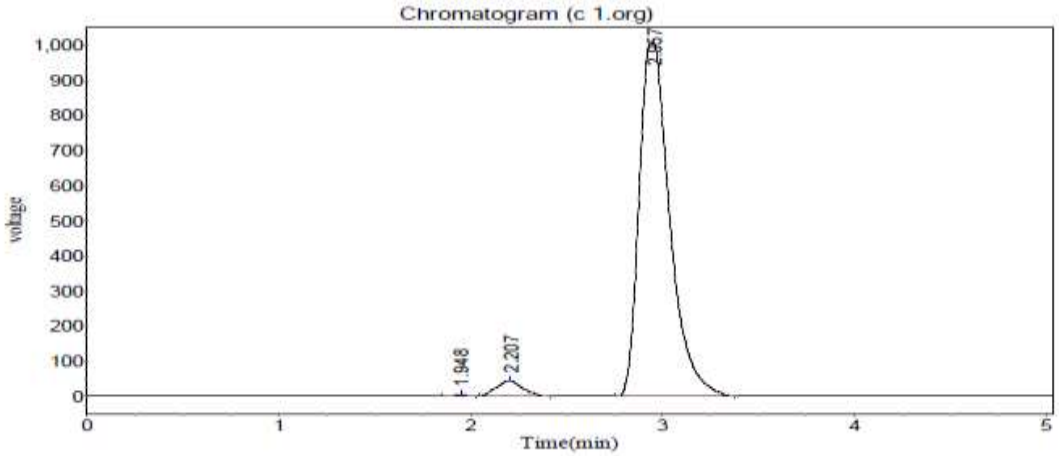


Fig.2 chromatographic trial 1

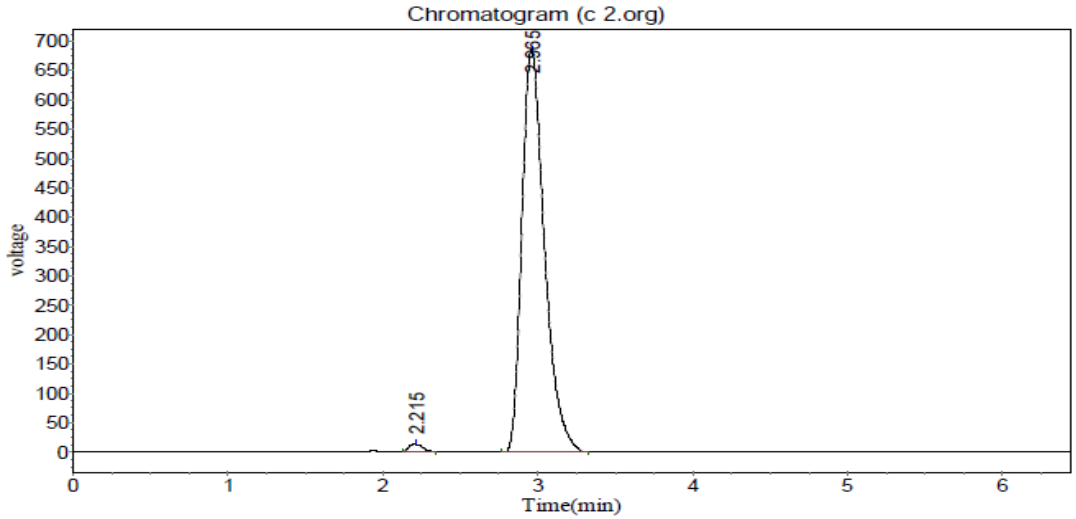


Fig.3 chromatographic trial 2

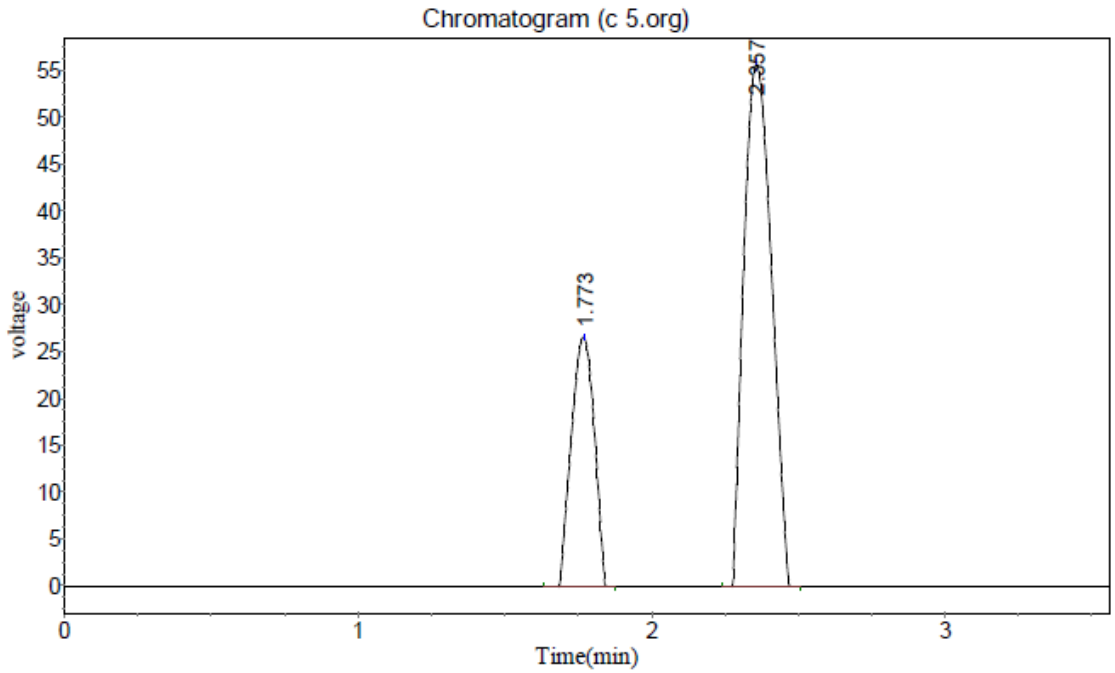


Fig.4 chromatographic trial 3

Table 5 standard assay results

INJECTION	CILNIDIPINE AREA		
	Area	Plate count	Tailing factor
Injection1	1716125	2187.076	1.288
Injection2	1713528	2051.078	1.208
Injection3	1761301	2239.807	1.297
Average	1730318		
Standard Deviation	26863.47		
% RSD	1.552516		

SAMPLE CHROMATOGRAMS-

Table 6 sample assay results

INJECTION	CILNIDIPINE AREA		
	Area	Plate count	Tailing factor
Injection1	1708454	2235	1.33
Injection2	1735796	2458	1.26
Injection3	1723413	2320	1.59
Average	1722554.333		
Standard Deviation	13691.20967		
% RSD	0.2226		

Calculation: CILNIDIPINE

Assay Results: (cilnidipine)

$$\text{ASSAY \%} = \frac{172254 * 10 * 666.666 * 99.58 * 1659}{1730318 * 666.666 * 1659 * 100 * 10} \text{ X } 100 = 99.13318$$

The results obtained for cilnidipine was found to be 99.133 %. Hence the results were within the limits.

System Suitability Results:

1). Tailing factor obtained from the standard injection was 1.264

2). Theoretical plates obtained from the standard injection were 2159.320

METHOD VALIDATION:

Accuracy-

Accuracy is measured in drug products by spiking known amounts (50%, 100%, and 150 %) of the analyte into the excipients and calculating the percent recovered.

Table 7 -Accuracy results of cilnidipine

Accuracy 50%		Accuracy 100%		Accuracy 150%	
Injection	Cilnidipine area	Injection	Cilnidipine area	Injection	Cilnidipine area
Injection1	2598822	Injection1	3403387	Injection1	4338611
Injection2	2598863	Injection2	3438509	Injection2	4372355
Injection3	2582457	Injection3	3475464	Injection3	4378742
Average	2593381	Average	3439120	Average	4363236
Std. Dev.	9460.2	Std. Dev.	36042.4	Std. Dev.	21563.7
% RSD	0.36478	% RSD	1.04801	% RSD	0.49421

Table 8 -Accuracy results of cilnidipine

% Concentration (at specification Level)	Area	Amount Added (µg)	Amount Found (µg)	% Recovery	Mean Recovery
50 %	2593381	22.5	22.481833	99.74667	99.04%
100 %	3439120	30	29.81348	98.73333	
150 %	4363236	37.5	37.82457	98.64	

The accuracy studies were shown as % recovery for cilnidipine at 50 %, 100 % and 150 %.The limits of % recovered should be in range of 98-102 % the results obtained for cilnidipine were found to be within the limits. Hence the method was found to be accurate and also reveal that the excipients present in the formulation did not interfere in the proposed method.

Precision:

The %RSD for the area of five replicate injections was found to be within the specified limits.

Table 9: Observation of System Precision

Injection	Injection1	Injection2	Injection3	Injection4	Injection5	Average	Standard Deviation	% RSD
Cilnidipine area	1681853	1708454	1672787	1676413	1735796	1695061	26725.7	1.57668

The acceptance limit should be not more than 2 % and the results obtained for standard samples (method precision) were found to be with in the acceptance limits i.e. 1.57. This indicates that the method has good repeatability.

INTERMEDIATE PRECISION:

For Intermediate precision studies 5 replicate injections formulation (method id precision) was performed. % RSD was determined for peak areas of sample of cilnidipine. The acceptance limit should be not more than 2 % and the result obtained for sample (method ID precision) was found to be with in the acceptance limits. This indicates that the method has good reproducibility.

Table10: Observation of intermediate system Precision

Injection	Injection1	Injection2	Injection3	Injection4	Injection5	Mean	Standard Deviation	% RSD
Cilnidipine area	1723413	1741466	1768845	1797206	1762878	1758762	28041.3	1.59438

LINEARITY

Table No.11: Linearity observation of CILNIDIPINE

S. No	Linearity Level	Concentration	Area
1	I	5 ppm	348228
2	II	10 ppm	984296
3	III	15 ppm	1689819
4	IV	20 ppm	2380880
5	V	25 ppm	2929908
Correlation Coefficient			0.999183

Acceptance Criteria: Correlation coefficient should be not less than 0.9

The linearity range was found to be 5-25µg/ml for cilnidipine. Calibration curve was plotted and correlation co-efficient for the drug found to be 0.999. Hence the results obtained were within the limits.

Limit of Detection:

The limit of detection was calculated from Slope and Standard deviation of response and LOD limit of cilnidipine was found to be 0.61 µg/ml. This indicates minimum level at which the Analyte was detected.

Limit of Quantitation:

The limit of quantification was calculated from Slope and Standard deviation of response and LOQ limit of cilnidipine was found to be 2.04 µg/ml. This indicates minimum level at which the analyte was quantified with acceptable accuracy and precision.

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, was made to evaluate the impact on the method.

On evaluation of the results, it can be concluded that the variation in flow rate has not affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10 %.

The method is robust only in flow condition. The results are reported in table number 12.

a) Test of robustness by deliberate change in the flow rate

Table 12: Flow rate observation of cilnidipine

S.No	Flow rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9ml/min	2155.130	1.221
2	1 ml/min	2155.420	1.208
3	1.2ml/min	2156.740	1.291

On evaluation of the above results, it can be concluded that the variation in flow rate did not affect the method significantly.

b) MOBILE PHASE: Robustness results for change in mobile phase composition

Table 13 : Change in mobile phase observation of cilnidipine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2362.003	1.292
2	*Actual	2252.420	1.294
3	10% more	2184.406	1.296

For robustness studies the chromatograms were recorded by changing the flow rate ± 0.1 ml/min and in mobile phase organic ratio ±10%. On evaluation of the results, it can be concluded that the variation in 10 % Organic composition in the mobile phase has not affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase.

Robustness studies reveal that the method was reliable.

System suitability parameters:

Table 14 Observation of System Suitability Parameters

S. No	Parameter	CILNIDIPINE
1	Retention time	2.79
2	Theoretical plates	2159.320
3	Tailing factor	1.264
4	Area	1730318

The system suitability Parameters were found to be within the specified limits for the proposed method

SUMMARY FOR RP-HPLC

Table 15: Summary for RP-HPLC Method

S.NO	Parameter	Acceptance criteria	Results obtained
1	System suitability	Theoretical Plates-NLT2000	2159.32
		Tailing factor-NMT 2	1.264
2	Precision	% RSD	1.57
3	ID Precision	% RSD	1.59
6	Linearity	Correlation coefficient NLT 0.999	0.999183
7	Accuracy	Percentage Recovery 98-102%	98.7333
8	LOD	0.61 µg/ml	
9	LOQ	2.04 µg/ml	

CONCLUSION:-

The proposed HPLC method was found to be specific, precise, accurate, rapid and economical for estimation of cilnidipine in Pharmaceutical dosage form. A Symmetry C18 column (4.6 x 150mm), with mobile phase containing ortho phosphoric acid Buffer pH 4 and Acetonitrile in 60:40 v/v was used. The use of this mobile phase resulted in peak with good shape and resolution. The flow rate was 1 ml/min and the analyte was monitored at 240nm wavelength. The retention time for cilnidipine was found to be 2.35 min

The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness and results were validated statistically according to ICH guidelines[7]. The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine Analysis.

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