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A Validated Stability Indicating RP-HPLC Method for Quantification of Cilnidipine in Bulk and in Tablet Dosage Form

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ABSTRACT

A stability indicating RP-HPLC method has been developed for quantification of Cilnidipine in bulk and in tablet dosage form. The chromatographic analysis was accomplished at ambient temperature on Xttera RP18 (100 x 4.6 mm, 3.5 μm) column and 1 mL/min flow rate by using Eluent composed of 10 mM phosphate buffer pH 2.6 with Acetonitrile (300:700, v/v). The UV detection at the wavelength of 240 nm was carried out using 20 μL injection volume. The Cilnidipine retention time was found to be 3.029 minute. The method in the range of 40.0573-120.1719 μg/mL was found to be linear (R^2 = 0.999) with a detection limit and quantitation limit of 1.2038 and 3.6478 µg/mL, respectively. The mean recovery% over the three tested levels of 50, 100 and 150% were found to be 98.74, 99.60, and 98.23%, respectively. The mean %assay of 99.29 for method repeatability and 98.82 for intermediate precision were found with %RSD of 0.68 and 0.31, respectively. Cilnidipine drug substance and their product exposed to acid, alkali, oxidative, thermal, photolytic and humidity stress conditions. The acid, alkali and photolytic induced stress studies signifying the formation of a variety of degradants and their peaks were well resolved from that of active analyte peak. Hence, it is recommended that the Cilnidipine drug substance, as well as drug product, should be store in a tightly closed container protected from light. The method as per ICH guidelines was validated for specificity, linearity, detection limit, quantitation limit, precision, accuracy, robustness, solution stability, and can be effectively used for routine analysis.

Keywords: RP-HPLC, Cilnidipine, Forced Degradation, Solution stability, Validation.

INTRODUCTION

Chemically, Cilnidipine is 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-Pyridine dicarboxylic acid 2-methoxyethyl (2E)-3-phenyl-2-propenyl ester (Fig. 1). It is a light yellow, crystalline powder and official in Indian Pharmacopoeia. Cilnidipine is a dihydropyridine derivative of 4th generation Ca^{++} channel blocker developed by Fuji Viscera Pharmaceutical Company and Ajinomoto, Japan and used for the treatment of hypertension. It is approved in Japan, India, China, Korea and some of the European countries.[1-3]

Literature review shows that few methods are reported for the determination of Cilnidipine by

HPLC[4-7] technique. The reported HPLC methods have certain limitations such as difficult solution preparation procedures and long run time. Among the reported methods, some method reports about forced degradation study, that too with only mild stress condition on the drug substance only but not on drug product. Also, solution stability of standard, sample, and mobile phase along with few robustness parameters are needed to be performed. Hence, present work endeavours to perform forced degradation study at more harsh stress condition on drug substance as well as drug product for the development of validated stability indicating RP-HPLC method for the Cilnidipine estimation which will be more simple, sensitive, rapid, precise, accurate and robust enough.

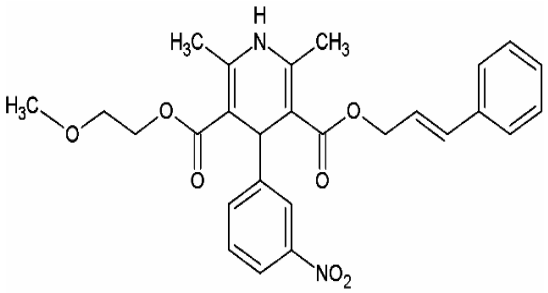

Fig. 1. Structure of Cilnidipine

Stress testing used to illustrated the inherent stability features of the active component.[8] Related substances are generated as degradants from improper handling or storage and/or as impurities from manufacturing process or as metabolites that may be inactive, active or sometime toxic and affecting the results of quality, safety and efficacy. The method capable to resolves degradation products or impurities from the active component has considered as good stability indicating approach.[9-12]

MATERIALS AND METHODS

Materials

Alkem Laboratories Ltd., Mumbai-Maharashtra (India) provided a free sample of pure drug Cilnidipine (Batch no.: JR/CLN/FP/17006, Assay: 99.25%). The chemicals like Ultrapure Water, ortho-Phosphoric Acid (OPA), Potassium Dihydrogen Phosphate (PDP), Acetonitrile (ACN), Sodium Hydroxide (NaOH), 6% v/v Hydrogen Peroxide (6% v/v H_2O_2), Hydrochloric Acid (HCl), Methanol (MeOH), etc of HPLC grade or equivalent were utilized for the study. CILACAR® 10–each film-coated tablets contain Cilnidipine 10 mg was procured from the local market (Batch no.: KC919095, Make: J. B. Chemicals and Pharmaceuticals Ltd., Daman–India).

Instruments

Materials weighed by using an analytical weighing balance (Model: CY204, Make: Citizon). Ultrasonic Bath (Model: LMUC-3, Manufacturer: Labman Scientific Instruments) was employed for the sonication. The estimation of solution pH was done by using Digital pH meter (Model: LT-49, Make: Labtronic Laboratory Instruments). Refrigerator (Model: GL-A282SPZL, Make: LG) was also utilized for solution stability study. The Photostability Chamber (Model: SRL-PHSC-11-A, Make: SR Lab Instruments India Pvt. Ltd.), Hot Air Oven (Model: BTI-29, Make: Bio-Technics India) and Stability Chamber (Model: GMP, Make: Labline Stock Centre) were used during the forced degradation study. Ultrapure Water was collected from Water Purification System during experimental work (Model: WPS211; Make: Analytical Technologies Limited). The method was developed by using Shimadzu HPLC (SCL-10Avp) having 20 µL rheodyne sample injector and UV detector. Cilnidipine elution was performed using Xttera RP18 (100 x 4.6 mm, 3.5 μm) column having P/N: 186000438 (Make: Waters) as stationary phase. LCsolution version 1.25 software was employed to control the chromatographic system and also for the data collection and data processing.

Chromatographic conditions

Chromatographic conditions used for Cilnidipine analysis are mentioned in Table 1.

Table 1: Chromatographic conditions

Parameters		Description
Instrument Name	:	HPLC
Detector	:	UV
Eluent (Mobile Phase)	:	10 mM phosphate buffer pH 2.6 and Acetonitrile (300 : 700, v/v)
Column	:	Xttera RP18 (100 x 4.6 mm, 3.5 μm) column (P/N: 186000438, Make: Waters)
Pump mode	:	Isocratic
Detection Wavelength	:	240 nm
Column temperature	:	Ambient (about 25°C)
Volume of Injection	:	20 μL
Flow rate	:	1.0 mL/min
Run time	:	6 min
Diluent/ Solvent (Blank)	:	Mobile Phase

Preparation of 10 mM phosphate buffer pH 2.6

Weighed and transferred 1.36 g of PDP into 1000 mL of water. Sonicated for 10 min to dissolve and pH 2.6 adjusted with 5% OPA and further filtered by utilizing 0.45 μm Nylon membrane filter (Manufacturer: Advanced Microdevices Pvt. Ltd.) under vacuum filtration.

Mobile phase preparation

Mixed pH 2.6 phosphate buffer (10 mM) and ACN (300 : 700, v/v) and sonicated for 10 min to degas.

Standard solution preparation

Weighed Cilnidipine standard (20 mg) and subsequently added into a 50 mL volumetric flask. To this, 35 mL of diluent was added and sonicated the solution for 5 min by intermittent shaking to dissolve the content. Kept this prepared solution on bench top to reach room temperature (RT) and up to the mark, filled with diluent, and mixed well (Cilnidipine concentration = 400 µg/mL).

Transferred exactly 5 mL of this produced stock solution of the standard into a 25 mL volumetric flask and with diluent, made up to mark. Mixed well and used this solution as a working standard solution (Cilnidipine concentration = 80 µg/mL).

The suitability of standard was confirmed with duplicate standard preparations.

Sample solution preparation

Weighed 20 Cilnidipine Tablets 10 mg (CILACAR® 10) to determine the average weight, and subsequently transferred 248.6 mg powdered tablets (equivalent to 20 mg of Cilnidipine) into a 50 mL volumetric flask. To this, 35 mL of diluent was added and sonicated the solution for 15 min by intermittent shaking to dissolve the content. Kept this prepared solution on bench top to reach room temperature and up to the mark, filled with diluent, and mixed well. Whatman filter paper (Manufacturer: GE Healthcare UK Ltd.) was utilized to filter this solution by discarding 5 mL of the earliest filtrate.

Transferred exactly 5 mL of this produced stock solution of sample into a 25 mL volumetric flask and with diluent, made up to mark. Mixed well and used this solution as the working sample solution.

Method validation

According to ICH guidelines Q2 (R1), the proposed chromatographic method was validated.[13]

Specificity

For this, interference was checked at the retention time of the Cilnidipine peak from the blank solution. In addition to this, specificity was studied in a forced degradation study with isocratic elution mode by doubling the run time to check any late eluting degradant peak. In this study, forced degradation was carried out by subjecting known concentration of Cilnidipine drug substance (API) as well as drug product sample (CILACAR® 10) to various stress conditions like acid (2N HCl, 3 h at RT), alkali (2N NaOH, 3 h at RT), oxidative (6% v/v, H_2O_2, 3 h at RT), thermal (70^{0}C in a hot air oven for 24 h), photolytic (UV light in Photostability Chamber for 24 h) and humidity (75% RH in Stability Chamber for 48 h) degradations. Similarly, blank solutions (without active components) were prepared for acid, alkali, and oxidative stress conditions to verify no interference at Cilnidipine retention time. However, the proposed RP-HPLC method was used to analyze all stressed samples, and results for %assay and %degradant (mass balance) were determined against the standard and judged with the unstressed sample.

System Repeatability and System Suitability

The parameters of system suitability were checked from 1st injection of standard solution. To check system reproducibility, %RSD was determined from five replicates injection of standard solution 1. Also, injected the one replicate of standard solution 2 and %relative difference between two standard solutions was calculated to confirm the suitability of standard.

Linearity

Linearity solutions for Cilnidipine in the range of 40.0573-120.1719 μg/mL were prepared from standard stock (concentration = 2002.8650 µg/mL) solution and established with different five concentrations from 50 to 150 %levels to nominal working concentration. The squared correlation coefficient (R^2) was determined from the linearity plot recorded for concentration vs. peak area response.

Detection Limit (DL) and Quantitation Limit (QL)

The DL and QL of Cilnidipine were derived based on the standard deviation of the response (residual value) and the slope method.

It was calculated as per ICH guidelines from the calibration curve of Cilnidipine by using the below equations.

$$DL = \frac{3.3 \times \sigma}{S} \quad \text{and} \quad QL = \frac{10 \times \sigma}{S}$$

Where, σ = the standard deviation of the response; S = the slope of the calibration curve.

Accuracy

Accuracy was evaluated by analyses of triplicate samples at three concentrations levels 50, 100, and 150% of nominal working concentration which containing a placebo mixture with Cilnidipine. Recovery results were calculated by injecting each sample once into the chromatographic system, and the mean recovery% at each level for triplicate samples was reported.

Precision

Precision should be studied using a homogeneous sample of Cilnidipine for assay determination.

Method Repeatability

It was performed by injecting 6 sample solutions prepared independently for Cilnidipine Tablets 10 mg using batch no. KC919095 (Make: J. B. Chemicals and Pharmaceuticals Ltd., Daman–India) as per the developed method. Also, results of system repeatability and system suitability were determined. Results were calculated for Assay percentage, %RSD, and 95% confidence interval.

Intermediate Precision

It was performed on 6 sample solutions prepared independently of the same sample of Cilnidipine Tablets 10 mg analyzed in Method Repeatability on a different day by the different analyst. Also, results of system repeatability and system suitability were determined. Results were calculated for Assay percentage, %RSD, and 95% confidence interval. In addition to this, %relative difference was determined between results of Method Repeatability (MR) and Intermediate Precision (IP).

Robustness

The filter compatibility for Cilnidipine Tablets 10 mg was established on triplicate sample preparations and divided all sample preparation into 3 parts. As per method, Whatman filter (Manufacturer: GE Healthcare UK Ltd.) was used to filter one part by discarding first 5 mL filtrate. The 0.45 μm Nylon Syringe filter (Manufacturer: Advanced Microdevices Pvt. Ltd.) was used to filter second part by discarding the first 5 mL filtrate and the 0.45 μm PVDF Syringe filter (Manufacturer: Advanced Microdevices Pvt. Ltd.) was used to filter third part by discarding first 5 mL filtrate. At last, results were calculated for %assay and %relative difference.

The changes in sonication time from 10 min to 20 min were performed to check the extraction efficiency of the method for sample preparation. This was demonstrated by using 3 replicate sample preparations of CILACAR® 10 for every changed condition. At last, the results were calculated for %assay and %relative difference.

Robustness was performed by making a little variation in chromatographic parameters like flow rate (± 0.1 mL/min); composition of mobile phase buffer (± 10%); mobile phase buffer pH (± 0.2); PDP quantity changed in mobile phase buffer (± 10%) and assessed the impact of every altered condition on the method. The results of system repeatability and system suitability were verified for all robustness parameters.

Stability of Solution

The solution stability of standard was checked after day 1 and day 2 storage in the refrigerator (2–8°C) and at room temperature on duplicate preparations. The percentage relative difference was calculated among the peak area observed from freshly prepared and stored standard solution.

The sample solution stability was checked after day 1 and day 2 storage at the refrigerator (2–8°C) and at room temperature on triplicate sample preparations. The %relative difference between stored and initial sample solutions (%assay) was calculated.

The mobile phase stored at bench top (room temperature) was evaluated after day 1 and day 2 for their stability. The appearance, system repeatability, and system suitability parameters were observed at the time of evaluation.

Range

The range of method is established based on precision, accuracy, and linearity data. Linearity, as well as accuracy for Cilnidipine was verified from 50 to 150% of the nominal working concentration.

RESULT AND DISCUSSION

Chromatographic Method Development and Optimization

The parameters like specificity, linearity, accuracy, range, precision, and robustness were considered during RP-HPLC method development and validation for Cilnidipine in bulk and in tablet dosage form. The several different mobile phase (Eluent) compositions were undertaken to select the appropriate mobile phase and subsequently flow rate and working wavelength optimization were performed. Also, method optimization was made by using changed columns, ODS Hypersil, 250 x 4.0 mm, 5 μm (Make: Thermo Scientific, P/N: 30105-254030), Xttera RP18, 100 x 4.6 mm, 3.5 μm (Make: Waters; P/N: 186000438) and Oyster ODS3, 150 x 4.6 mm, 5 μm (Make: Merck, P/N: S670153). The ACN and MeOH were utilized as organic modifiers with Acetate buffer (10 mM) pH 4.5 and phosphate buffer (10 mM) at pH 3.0, 2.6 to get the best peak shape.

At last, the mobile phase (Eluent) comprised by phosphate buffer (10 mM) pH 2.6 ± 0.05 (adjusted with 5% OPA) and ACN in the ratio of 300:700, v/v was selected for Cilnidipine because it retained the peak of Cilnidipine in a short period with satisfactory number of theoretical plates and tailing factor. UV detection at 240 nm was used to record response with flow rate 1.0 mL/min at ambient condition (about 25°C) and 20 μL of injection volume. All calculations for quantitative assay of Cilnidipine were made on the basis of peak area.

Method Validation

Specificity

Interference was not observed from the blank at the retention time of Cilnidipine peak indicating the method specificity. The representative chromatograms of blank, Cilnidipine standard and sample are given in Figure 2.

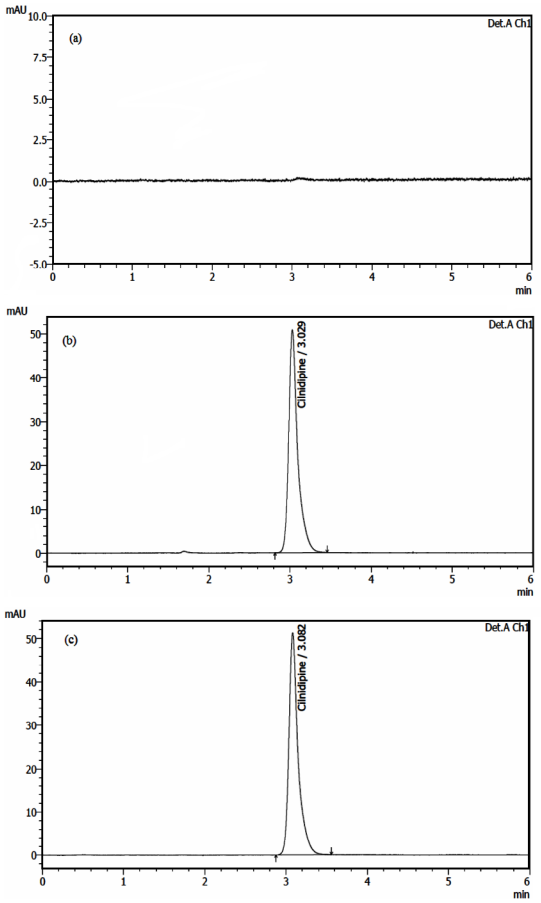

Fig. 2. Representative chromatograms of (a) Blank, (b) Standard, and (c) Sample

The forced degradation results shows that interference was not found at the retention time of Cilnidipine peak from the degradant peaks. Cilnidipine drug substance solution mass balance data was clearly demonstrated that the response is decreased in acid and alkali stressed solution with increase in the response of peaks of degradant, and major degradant found at 1.756 and 1.741 min respectively. Cilnidipine drug product mass balance data clearly demonstrated that the response is decreased in acid, alkali, and photolytic stressed sample with increase in the degradant peaks response, and major degradant found at 1.418, 1.423 and 2.785 min respectively. This shows that Cilnidipine was stable to humidity, thermal and oxidative stressed conditions while unstable to acid, alkali, photolytic stress conditions (Table 2). Hence, it is recommended that the Cilnidipine drug substance as well as drug product should be store in a tightly closed container protected from light.

The chromatograms of degradation samples (Fig. 3 - Fig. 5) were demonstrating that degradant peak separated from the drug peak, shows method is specific.

Table 2: Forced degradation results

Sample name	Condition	%Assay		%Total degradation		%Mass Balance	
		DS	DP	DS	DP	DS	DP
Unstressed	As per test method	99.29	100.97	NTD	NTD	99.29	100.97
Acid stressed	2N HCl at RT for 3 h	97.77	99.39	1.15	0.29	98.92	99.68
Alkali stressed	2N NaOH at RT for 3 h	98.49	57.96	0.90	26.25	99.39	84.21
Oxidative stressed	6% H2O2 at RT for 3 h	100.96	99.76	NTD	NTD	100.96	99.76
Photolytic stressed	UV light for 24 h	98.52	97.22	NTD	1.21	98.52	98.43
Thermal stressed	70°C for 24 h in oven	99.93	100.31	NTD	NTD	99.93	100.31
Humidity stressed	75% RH for 48 h	100.55	101.29	NTD	NTD	100.55	101.29

NTD = not detected; RH = relative humidity; RT = room temperature; DS = drug substance; DP = drug product

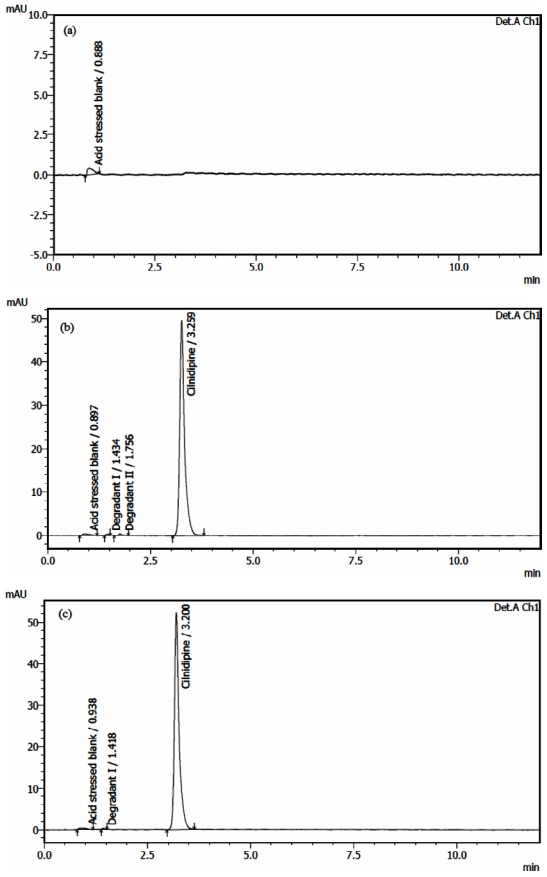

Fig. 3. Chromatograms of acid stressed (a) Blank; (b) Cilnidipine API; (c) Sample

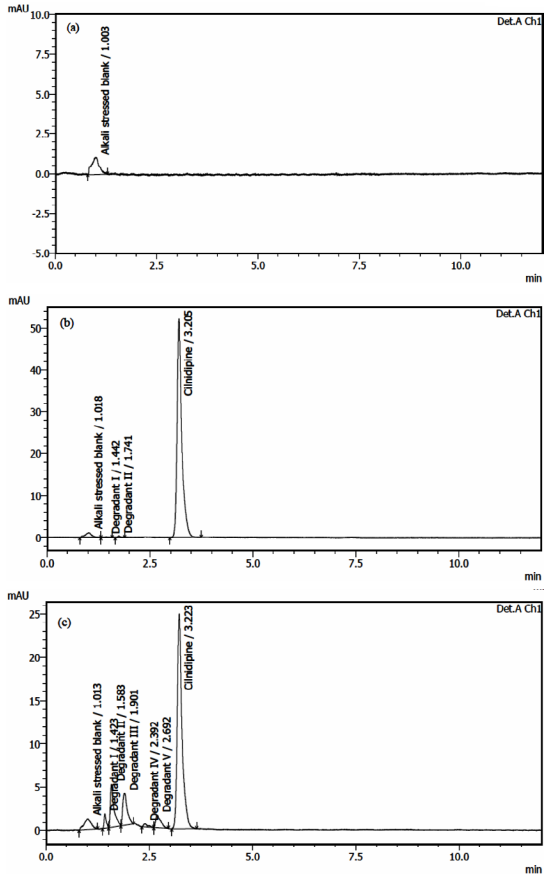

Fig. 4. Chromatograms of alkali stressed (a) Blank; (b) Cilnidipine API; (c) Sample

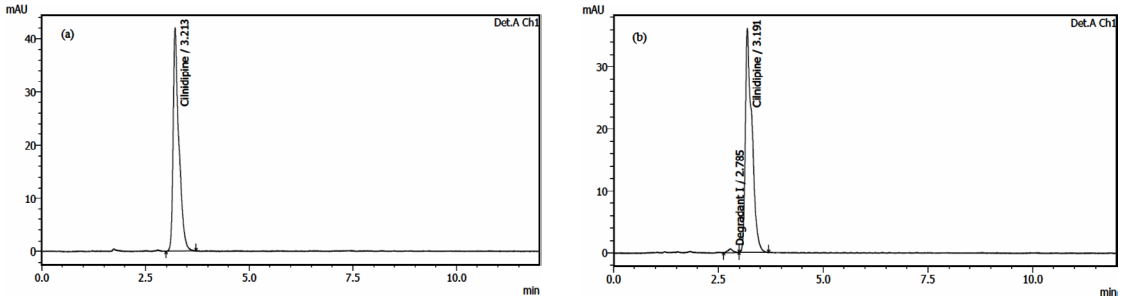

Fig. 5. Chromatograms of photolytic stressed (a) Cilnidipine API; (b) Sample

System Repeatability and System Suitability

Chromatographic system reproducibility features were established by system repeatability and system suitability parameters (Table 3).

Table 3: Results of system repeatability and system suitability

Parameters	Results	Acceptance criteria
Retention time (min)	3.029	NA
USP plate counts	4185	≥ 2000
USP tailing	1.22	0.8 – 2.0
%RSD found from injections of standard 1 (5 replicate)	0.40	≤ 2.0 %
The % relative difference between 2 different standard	0.77	≤ 2.0 %

Linearity

The method was linear for Cilnidipine from 40.0573-120.1719 μg/mL (R^2 = 0.999) over 50 to 150% level of nominal working concentration (Table 4). A result of linearity for Cilnidipine shows a good linear relationship in this studied range, representing the method fitness for analysis. Linearity graph of Cilnidipine is given in Figure 6.

Table 4: Results for Linearity

Level of Linearity (%)	Concentration (μg/mL)	Peak area
50	40.0573	196600
75	60.0860	299653
100	80.1146	396599
125	100.1433	499922
150	120.1719	603745
Squared correlation coefficient (R^2); ≥ 0.995		0.999
(Y-intercept/ peak area at 100% standard concentration) x 100; ≤ 3.0		1.64

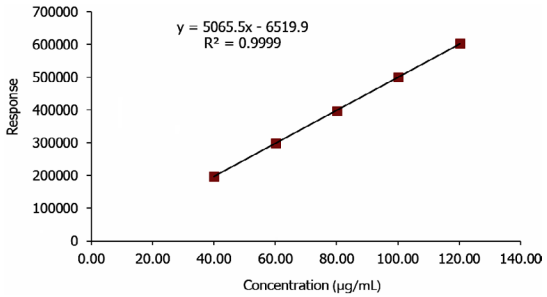

Fig. 6. Linearity graph of Cilnidipine

Detection Limit and Quantitation Limit

The Cilnidipine DL was 1.2038 μg/mL, shows that even little amount of the drug can be detected. The Cilnidipine QL was 3.6478 μg/mL, shows that even little amount of the drug can be quantified.

Table 5: Accuracy results

Accuracy level (%)	Concentration (μg/mL)	Average %Recovery*	%RSD
50	40	98.74	0.43
100	80	99.60	0.62
150	120	98.23	0.33

*Average of three replicate

Accuracy

The results of average %recovery for Cilnidipine were found to be 98.74, 99.60, and 98.23% at 50, 100, and 150 %levels respectively of nominal working concentration (Table 5). This shows the accuracy of the method and the excipients have no interference in the determination.

Precision

Results of MR and IP study illustrated that the %RSD values for Cilnidipine were less than 2.0 (Table 6), indicating that the method is reproducible as well as precise.

Table 6: Precision results

Sample No.	Weight of sample (mg)		Peak area		% Assay	
	MR	IP	MR	IP	MR	IP
1	247.1	248.1	401601	397420	99.43	98.38
2	248.8	247.9	400605	399312	98.50	98.93
3	246.7	249.6	398761	402495	98.88	99.04
4	248.2	248.4	405543	401324	99.96	99.23
5	249.6	249.2	403134	400572	98.81	98.72
6	245.9	247.5	402636	397528	100.17	98.65
Average	–	–	–	–	99.29	98.82
% RSD	–	–	–	–	0.68	0.31
% Relative Difference	–	–	–	–	–	0.47
95 % CI	–	–	–	–	98.75–99.83	98.58–99.06

95 % CI = 95 % Confidence Interval

Robustness

Results of sample filtered through 0.45 μm Nylon Syringe filter and 0.45 μm PVDF Syringe filter were met the %relative difference criteria (≤ 3.0 %) with results obtained with Whatman filter paper (Table 7). Hence, apart from Whatman filter paper, the 0.45 μm Nylon Syringe filter and 0.45 μm PVDF Syringe filter are also suitable for the assay samples filtration.

Table 7: Results for changed filter

Name of Filter	Sample	Assay (%)	% RD
Whatman filter (as per method)	1	99.96	NA
	2	98.81	NA
	3	100.17	NA
0.45 μm Nylon Syringe filter	1	99.23	0.74
	2	98.30	0.52
	3	99.74	0.43
0.45 μm PVDF Syringe filter	1	98.56	1.41
	2	98.20	0.62
	3	100.52	0.34

RD = Relative Difference; NA = Not applicable

Table 8: Robustness results for changed sonication time

Sample sonication (min)	Change in time (min)	Sample	Assay(%)	Average	%Relative difference
15(As per method)	0	1	99.43	98.94	NA
		2	98.50		
		3	98.88		
10	- 5	1	98.76	99.13	0.20
		2	100.22		
		3	98.42		
20	+ 5	1	100.06	99.41	0.48
		2	99.74		
		3	98.43		

The results obtained by varying time of sonication for preparation of sample from 10 min to 20 min were not affected for Cilnidipine Tablets 10 mg (Table 8) and met the acceptance criteria for %relative difference (≤ 3.0 %).

The results were met the acceptance criteria for system repeatability, system suitability, and retention time variation (Table 9) for each changed parameter, indicating its robustness.

Table 9: Robustness results for changed chromatographic conditions

Chromatographic conditions variation	Retention time (min)	Tailing factor	Plate count	% RSD	Retention time in min from sample
As per method	3.206	1.23	4396	0.51	3.022
Flow rate – 0.1 mL/min	3.339	1.24	4535	0.33	3.434
Flow rate + 0.1 mL/min	2.815	1.21	4152	0.66	2.824
Buffer phase – 10 %	2.825	1.28	4072	0.92	2.785
Buffer phase + 10 %	3.436	1.24	4512	1.50	3.392
Buffer pH – 0.2	3.045	1.23	4248	1.74	3.195
Buffer pH + 0.2	3.126	1.20	4263	0.48	3.086
Quantity of PDP – 10 %	3.357	1.25	4267	0.54	3.197
Quantity of PDP + 10 %	3.210	1.21	4161	0.22	3.197
Acceptance criteria	NA	0.8–2.0	≥ 2000	≤ 2.0	Similar to standard

Solution stability

The standard solution was found to be stable up to 2 days (Table 10) in the refrigerator (2-8°C) and at room temperature and the %relative difference (%RD) met the acceptance criteria (≤ 2.0%).

Table 10 : Stability of standard solution

Time in Days	First standard solution			Second standard solution		
	Response/mg		% Relative Difference	Response/mg		% Relative Difference
	Fresh standard	Stored standard		Fresh standard	Stored standard	
			Room temperature			
Initial	20165.2970	NA	NA	20011.0784	NA	NA
1	20022.0906	20256.9802	1.16	20022.0906	20151.7157	0.64
2	19941.5975	20253.2673	1.54	19941.5975	19926.8137	0.07
			Refrigerator (2 – 8°C)			
Initial	20165.2970	NA	NA	20011.0784	NA	NA
1	20022.0906	20120.3465	0.49	20022.0906	19917.9902	0.52
2	19941.5975	19860.3465	0.41	19941.5975	19697.1078	1.24

The sample preparation was found to be stable for 2 days (Table 11) in the refrigerator (2-8°C) and at room temperature as the % relative difference (≤ 3.0%) met the acceptance criteria.

Table 11: Summarized results for sample solution stability

Time	Sample no.	Refrigerator		Room Temperature	
		Assay (%)	% RD	Assay (%)	% RD
Initial	1	99.43	NA	99.43	NA
	2	98.50	NA	98.50	NA
	3	98.88	NA	98.88	NA
Day 1	1	100.45	1.02	99.83	0.40
	2	99.81	1.32	100.22	1.74
	3	101.07	2.19	99.75	0.88
Day 2	1	99.70	0.27	99.20	0.24
	2	99.48	0.99	99.27	0.77
	3	99.59	0.71	99.36	0.48

The mobile phase preparation was found to be stable for day 2 at room temperature as its clear appearance was found and met the acceptance criteria of system repeatability and system suitability during the evaluation of stability (Table 12).

Table 12: Results for mobile phase stability

Time	Working standard solution				Retention time in min from sample
	Retention time (min)	Tailing factor	Platecount	% RSD	
Initial	3.029	1.22	4185	0.40	3.082
Day 1	3.128	1.27	4500	0.50	3.150
Day 2	3.106	1.28	4393	0.14	3.084
Acceptance criteria	NA	0.8 – 2.0	≥ 2000	≤ 2.0	Similar to standard

Range

The method range for Cilnidipine was found from 50 to 150% level of nominal working concentration derived from suitable precision, accuracy, and linearity results.

Comparison with reported methods for Cilnidipine estimation

The proposed research work is offering a variety of advantages like short run time of the component with simple solution preparations in comparison with earlier reported work. Hence, this method is more cost-effective as it needs less analysis time. Two standard preparations are used to prove the system repeatability and system suitability of the method during the analysis. Robustness for change in filter and change in sonication time are performed. Also, solution stability is reported for standard, sample and mobile phase preparations. In addition to this, forced degradation study was carried out at more harsh stress conditions on drug product and drug substance. The obtained validation results suggests that the proposed RP-HPLC method is found to be more simple, specific, rapid, linear, accurate, precise and robust enough in comparison with reported methods (Table 13).

Table 13: Comparison with reported methods for Cilnidipine estimation

Parameters	Safhi and Nagaraj, 2013[7]	Kadam *et al.*, 2015[6]	Ravi Shankar *et al.*, 2019[5]	Tiwari *et al.*, 2020[4]	Proposed Method
Type of system	HPLC with UV detector	HPLC with PDA detector	HPLC with UV detector	HPLC with UV detector	HPLC with UV detector
Mobile Phase (Eluent)	MeOH : Phosphate buffer (0.05 M) pH 3 (80:20, v/v)	MeOH, Sodium Dihydrogen Orthophosphate buffer (pH=3) and ACN (75:18:7 v/v/v)	ACN : MeOH (50:50, v/v)	MeOH : PDP buffer (50:50, v/v)	Phosphate buffer (10 mM) pH 2.6 and ACN (300:700, v/v)
Column	Grace C18 (4.6 x 250 mm)*	Kromasil C18 (4.6 x 250 mm, 5 µm)	Thermo Scientific C18 (4.6 x 250 mm, 5 µm)	Cosmosil (4.6 x 250 mm, 5 µm)	Waters Xterra RP18 column (4.6 x 100 mm, 3.5 μm)
Flow Rate	1.0 mL/ min	1.0 mL/min	1.0 mL/ min	1.0 mL/ min	1.0 mL/min
Temperature of Column	Room temperature	Ambient	25°C	Ambient	Ambient (about 25°C)
Detection Wavelength	254 nm	240 nm	242 nm	241 nm	240 nm
Pump Mode	Isocratic	Isocratic	Isocratic	Isocratic	Isocratic
Run Time	10 min	20 min	10 min	15 min	6 min
Advantage	1. Considered as stability indicatingmethod 2. Standard solution stability established	Considered as stability indicating method	--	--	1. Short run time 2. Easy solution preparations 3. Standard, sample and mobile phase preparation stability established 4. Robustness for change in filter and change in sonication time is performed 5. FD performed on DS and DP, hence considered as stability indicating Method
Shortcoming	1. FD performed only on DS not on DP as well as DS not on DP as well as for thermal stressed condition	1. FD performed only on DS not on DP 2. Long run time	1. FD not performed 2. Long run time	1. FD not performed 2. Long run time	Separation as well as degradation products structural depiction were not done
Applications	1. Estimation of Cilnidipine in bulk and in Tablets 2. Stability sample analysis	1. Estimation of Cilnidipine in bulk and in Tablets 2. Stability sample analysis	Estimation of Cilnidipine in bulk and in Tablets	Estimation of Cilnidipine	1. Estimation of Cilnidipine in bulk and in Tablets 2. Stability sample analysis

*Particle size not reported by Author; FD = Forced Degradation; DS = Drug Substance; DP = Drug Product; NA = Not applicable

CONCLUSION

Stability indicating method for quantification of Cilnidipine in bulk and in tablet formulation was developed and validated by using RP-HPLC technique as per ICH guidelines. The RP-HPLC method results are linear in the proposed working concentration range as well as robust, accurate, precise, and specific. The results of forced degradation shows that the developed method is specific as well as stability indicating as peaks observed due to the degradants were distinguishable from the active analyte peaks. The solution stability results proved that preparations of standard, sample, and mobile phase can be used up to 2 days. Also, the easy solution preparations and simple isocratic elution offered cost–effective and rapid analysis of the drug. This method can be utilized in the quality control for regular analysis as well as stability studies of Cilnidipine in bulk and in their dosage form.

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Conflicts of interest

The authors report no conflict of interest.

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