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Research Article

Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Cilnidipine

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ABSTRACT

Cilnidipine is one of the dihydropyridine calcium antagonists. It was created combinedly by Fuji Viscera Pharmaceutical Company, Ajinomoto and Japan and was approved in the year 1995. Cilnidipine acts on N-type calcium channel where exist the end of sympathetic nerve in addition to common L-type calcium channel like that of other calcium antagonists. China, Japan, India, Korea and several other countries approved this drug. The objective of the method validation is to demonstrate whether the method was suited for the intended purpose. The method was validated as per the ICH guidelines. The method was validated for linearity, precision (repeatability, intermediate precision), accuracy, specificity, robustness, limit of detection and limit of quantification. Cosmosil (4.6 X 250mm, 5 μ) column was used for separation. The selected wavelength for Cilnidipine was 241 nm. The mobile phase consists Methanol: Potassium dihydrogen phosphate buffer (50:50). Flow rate was delivered at 1.0 mL/min. Appropriate dilutions of standard stock solutions were prepared as per the get desired concentrations in the range of 100-500 mcg/ml. The RT obtained was 4.8165 minutes.

Keywords: Cilnidipine, UV spectroscopy, RP-HPLC, ICH

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INTRODUCTION:

Cilnidipine is one of the dihydropyridine calcium antagonists. It was created combinedly by Fuji Viscera Pharmaceutical Company, Ajinomoto and Japan and was approved in the year 1995. Cilnidipine acts on N-type calcium channel where exist the end of sympathetic nerve in addition to common L-type calcium channel like that of other calcium antagonists. China, Japan, India, Korea and several other countries approved this drug.¹

IUPAC nomenclature: 3-(2-methoxyethyl) 5-(2E)-3-phenylprop-2-en-1-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate

Literature review suggest few RP-HPLC, HPTLC, spectroscopic, stability indicating HPLC determinations were performed.²⁻¹⁵ The aim of the present study is to develop a simple, precise, accurate, sensitive HPLC method for the determination. After, doing in depth study in the present research work, it was found that the present research work is having various advantages over the previous work. The advantages include less retention times of the component,

with good resolution. The % RSD of robustness was found to be less. The results obtained from the validation suggest that the method was found to be precise, accurate, linear and robust enough and the method was also found to be economical.

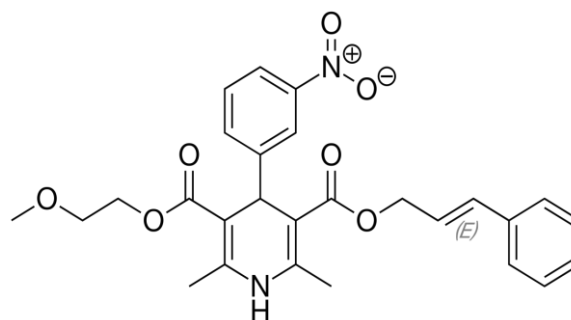


Figure 1: Structure of Cilnidipine

EXPERIMENTAL WORK:

Chemicals and reagents: HPLC grade acetonitrile, methanol, from Spectro Chemie. Ammonium acetate, acetic acid of analytical grade was used. Millipore grade water was used. The reference standard samples of Cilnidipine was provided was provided by Ajanta Pharma.

Instrumentation and analytical conditions:

The analysis was carried out by using Younglin (S.K) Gradient System UV Detector, 4.6X250mm cosmosil column, 20ml loop size HPLC (With UV/Vis Detector. Other instrumentation includes Double beam UV- Visible spectrophotometer Simadzu UV-1800, digital balance (metler toledo), vacuum pump (Gelman science), pH meter (poloman).

Chromatographic conditions:

Cosmosil (4.6X250mm, 5 μ) column was used for separation. Methanol: Potassium dihydrogen phosphate buffer (50:50) was the optimized mobile phase.

Preparation of Standard stock solution:

100 mg of Cilnidipine was weighed in to 100 ml volumetric flasks. The drug was dissolved in 40 ml of solvent, and shaken manually for 10 min. The volume was made up to the mark with solvent and the final strength obtained was 1000 μ g/ml.

Preparation of Working Standard Solutions:

10ml of the standard stock solution of Cilnidipine was pipette out in to 100 ml volumetric flasks and the volume was made up to the mark with solvent, and the final strength obtained was 100 μ g/ml.

Validation procedure:

Method validation has the objective of establishing the suitability of any method for the experimental purposes. Method validation are carried out as per the ICH guidelines. Linearity, precision (repeatability, intermediate precision), accuracy, specificity, robustness, limit of detection and limit of quantification were checked in the method validation. A calibration curve was constructed by taking six different concentrations. The peak area was calculated, and calibration curve was constructed by taking peak area and concentration on both the axis. The linearity was evaluated by linear regression analysis. The precision studies were demonstrated by two parameters inter day and intraday precision. Intraday precision was performed by injecting six replicated injections to the chromatographic system on the same day and calculated the %RSD. The inter day precision was performed by injecting six replicated injections at two consecutive days. From the peak area of the chromatograms, the %RSD was calculated. The accuracy was determined by adding a known amount of the standard to the sample, and the percentage recovery was estimated. The robustness was determined by incorporating deliberate changes into the method conditions like the change in flow rate, pH, and gradient.

RESULTS AND DISCUSSION:

Selection of wavelength:

UV spectrum was obtained by preparing a solution by taking diluents and scanned between 200 to 400 nm. Brinzolamide shows λ_{max} at 241 nm. So, it is selected as a detection wavelength.

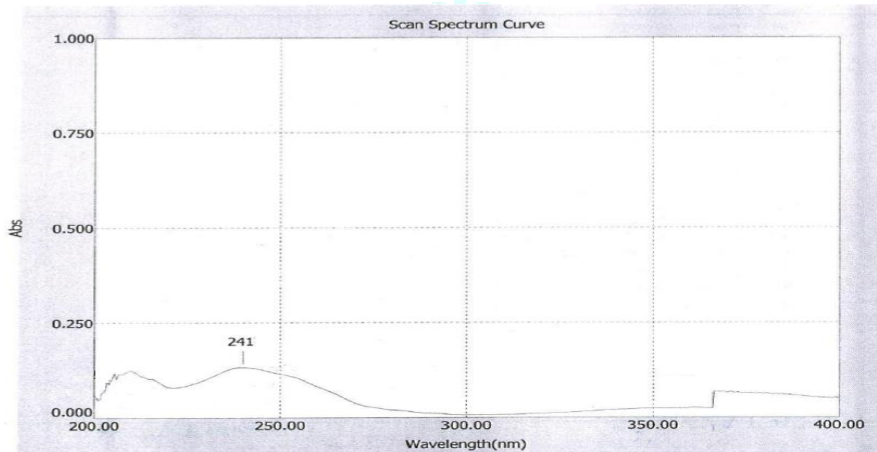


Figure 2: Spectrum of Cilnidipine

Standard curve of Cilnidipine:

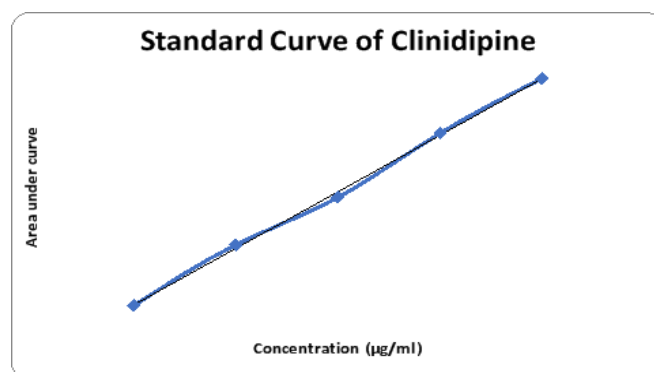


Figure 3: Standard curve of Cilnidipine

Development and optimization of the HPLC method:

For getting an optimized chromatographic condition, A Cosmosil (4.6X250mm, 5 μ) column was used for separation. The mobile phase consists of Methanol: Potassium

dihydrogen phosphate buffer (50:50). Flow rate was delivered at 1.0 mL/min with detection wavelength at 241 nm. 20 μ L was injected to the chromatographic system with ambient temperature. The RT obtained was 4.8165 minutes.

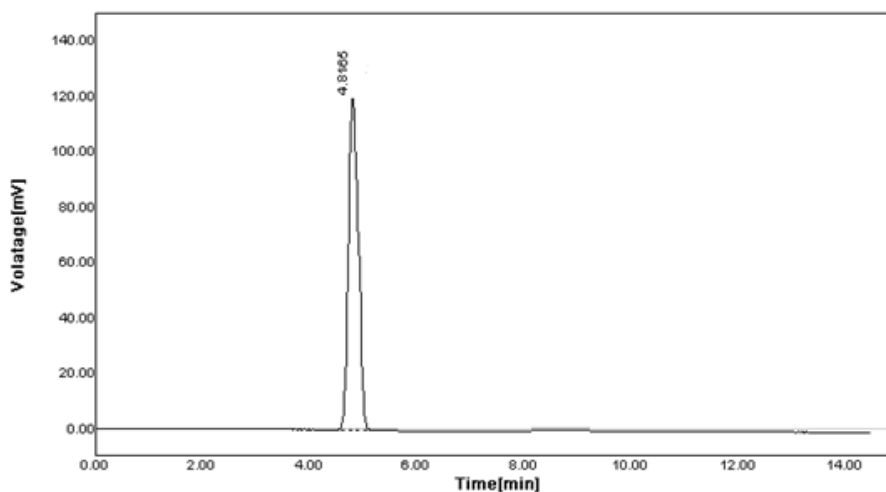


Figure 4: RT of Brinzolamide

Method validation:**Linearity:**

Appropriate dilutions of standard stock solutions were prepared to get desired concentrations in the range of 100-500 mcg/ml. Then the concentrations were plotted against the area under curves to get the equation of standard curve, as presented in Table 1.

Table 1: Dilutions for linearity study

Stock solution (μ l)	Diluted (ml)	Final concentration
100	10	10
200	10	20
300	10	30
400	10	40
500	10	50

Table 2: Linearity Data

Sr. No.	Conc. in (μ g/ml)	Mean AUC (n = 5)	%RSD
1	10	4198	0.567
2	20	8813	0.493
3	30	12412	0.681
4	40	17289	0.392
5	50	21432	0.764

Precision:

Precision is determined at two levels: a) Repeatability b) Intraday and Interday Precision.

Table 3: Precision Data

Parameter	Amount taken (μ g/ml)	Amount found* (μ g/ml)	%RSD
System Precision	60	59.91	0.681
Method Precision	60	60.12	0.592

*Mean of Six observations

Table 4: Intraday and Interday Precision Data

Concentration of Brinzolamide	Intraday*	%RSD	Interday*	%RSD
20 μ g/ml	20.09	0.118	19.92	0.249
40 μ g/ml	39.92	0.132	39.94	0.362
60 μ g/ml	60.13	0.209	59.91	0.419

*Mean of three observations

Accuracy:

To check the accuracy of the developed methods, analytical recovery experiment was carried out by standard addition method. Recovery study was performed by adding 80, 100 and 120 % of the test concentration as per ICH guidelines.

Table 5: Accuracy

Sr. No.	Concentration of Drug Added (µg/ml)	Concentration of Drug Added (µg/ml)	% Recovery± SD
1	64	10.4	99.99 ± 0.182
2	80	13	99.82 ± 0.273
3	96	15.6	98.77 ± 0.317

Limit of Quantification (LOQ) and Limit of Detection (LOD):

The limit of quantification (LOQ) is defined as the lower concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity. The LOD and LOQ were calculated using formula given below.

$$\text{LOD} = 3.3 \times \sigma / S$$

$$\text{LOQ} = 10 \times \sigma / S$$

Where, σ = Standard deviation of the Y- intercepts of the 5 calibration curves, S = Mean slope of the 5 calibration curves. The LOQ and LOD parameters of Brinzolamide are provided in Table 6.

Table 6: LOD and LOQ parameters

Parameter	Values
S.D. of Intercept*	0.6148
Mean Slope of Calibration Curve*	430.79
LOD (µg/ml)	0.00471
LOQ (µg/ml)	0.01427

Robustness:

Robustness was studied by observing the change in following parameters, and then observation of each parameter change was done to access their effect on system suitability and assay. Change in mobile phase composition was done by ± 5.0 ml of organic solvent and the change in the detection wavelength ± 10 nm was done.

Change in mobile phase composition: The sample solution at test concentration (60µg/ml) of drug was injected thrice with the mobile phase composition changed by ± 5.0 ml of organic solvent from the developed method.

Change in detection wavelength: The sample solution at test concentration (60µg/ml) of drug was injected thrice with the change in detection wavelength by ± 10 nm from the developed method. The % assay of drug after changes in method parameters were observed.

CONCLUSION:

A precise RP – HPLC method was developed for the determination of Brinzolamide. The shorter run time elutes Erlotinib hydrochloride with good resolution, and symmetry. The method was validated as per the ICH

guidelines and the method was found to be simple, precise, linear, accurate, rugged and robust enough.

Table 7: Summary of Validation parameters

Parameters	Cilnidipine
Accuracy	98.77 ± 0.317– 99.99 ± 0.182
Precision (%RSD)	
System Precision	0.681
Method Precision	0.592
Intra-day (n = 3)	0.118 - 0.209
Inter-day (n = 3)	0.249 - 0.419
LOD and LOQ	
LOD	0.00471
LOQ	0.01427

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