

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND OLMESARTAN MEDOXOMIL IN THEIR COMBINED TABLET DOSAGE FORM

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ABSTRACT

A Simple, Sensitive, Accurate and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of Olmesartan medoxomil and Cilnidipine. The proposed RP-HPLC method utilizes stationary phase consist of symmetry C18 (250 x 4.6mm, 5 µm in particle size) with a mobile phase comprising of Acetonitrile:Buffer (75:25 %v/v) pH 6.5 adjusted by 1 % Triethylamine at a flow rate of 1.0 ml/min, column temperature of 25°C and UV detection at 265nm. The retention time of Olmesartan medoxomil and Cilnidipine were 2.655 min and 4.720 min respectively. The linearity were found to be in the range of 10-90 µg/ml and 20-180 µg/ml for Cilnidipine and Olmesartan medoxomil respectively. The % Recovery were found to be 99.26 ± 0.226 for Cilnidipine and 99.51 ± 0.3030 for Olmesartan medoxomil for. The proposed method was validated as per ICH guidelines and successfully applied for the determination of drugs in tablet.

KEY WORDS

Cilnidipine, Olmesartan medoxomil, simultaneous estimation, validation, RP-HPLC.

INTRODUCTION

Cilnidipine O3-(2-methoxyethyl) O5-[(E)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate is a novel and unique dihydropyridine calcium channel blocker that possesses a slow-onset, long lasting vasodilating effect¹.

Olmesartan medoxomil, is a prodrug that works by blocking the binding of angiotensin II to the AT₁ receptors in vascular muscle; it is therefore independent of angiotensin II synthesis pathways, unlike ACE inhibitors. By blocking the binding rather than the synthesis of angiotensin II, Olmesartan inhibits the negative regulatory feedback on renin secretion. As a result of this blockage, Olmesartan reduces vasoconstriction and the secretion of aldosterone. This lowers blood pressure by producing vasodilation, and decreasing peripheral resistance.^{2,3}

Review of Literature revealed that, there are some methods available for estimation of Cilnidipine & Olmesartan medoxomil individually & in combination

with other drugs/dosage forms. But, still there is not a single method existing for simultaneous estimation of Cilnidipine & Olmesartan medoxomil combined dosage form. So, it is thought of interest to develop analytical method for estimation of both the drugs simultaneously in combined dosage forms.

MATERIALS AND METHODS

Drug and chemicals

All the chemical and reagents used were of analytical grade. Cilnidipine and Olmesartan medoxomil were obtained as gift sample from Pure Chem. Ankleshwar and Cadila Pharma, Ahmedabad. The combined dosage contains Cilnidipine 10 mg and Olmesartan medoxomil 20 mg of the drug was procured from local market.

HPLC apparatus

The separation was performed by using C-18 (250mm x 4.6mm, 5 µm). Column on a shimadzu LC

20 AT isocratic solvent delivery system .Shimadzu SPD-20A dual wavelength absorbance detector and Rheodyne injector. The mobile phase was freshly prepared ,filtered and sonicated before use and delivered at a flow rate of 1.0 ml/min and the detector wavelength was set at 265 nm.The injection volume was 20 µl.

Stock solution and standard

Standard stock solution was prepared for 1000 µg/ml by using Cilnidipine and Olmesartan medoxomil separated by using mobile phase. From the standard stock solution different concentration of working standard were prepared from the range of 10 to 90 µg/ml for Cilnidipine and 20 to 180 µg/ml for Olmesartan medoxomil.

Calibration curve

The calibration curve were constructed for the determination of linearity and the curves were plotted with the concentration range verses area must obey the Beer's law. The linearity was evaluated by the analysis of serially diluted sample in range of 10 to 90 µg/ml for Cilnidipine and 20 to 180 µg/ml for Olmesartan medoxomil. An aliquot was injected by using of methanol. The 20 µl mixture was injected for the estimation under the optimized chromatographic condition. The retention time of standard Olmesartan medoxomil and Cilnidipine were found to be 2.655 and 4.720 min respectively with a good resolution.

Analysis of formulation

Twenty tablets were weighted and finely powdered. A quantity equivalent to 10 mg Cilnidipine and 20 mg Olmesartan medoxomil were transferred into volumetric flask and dissolved in to mobile phase. The solution was ultrasonicated for 10 min and filtered

through 0.45 µ nylon membrane and degassed and the volume was made up to mark with same system. Above solution was taken to prepare a dilution of 100 µg/ml Cilnidipine and 200 µg/ml Olmesartan medoxomil . The amount of drug was determined and three replicate injection were done.

RESULT AND DISCUSSIONS

METHOD DEVELOPMENT

Several tests were performed in order to get satisfactory separation and the resolution of Cilnidipine and Olmesartan medoxomil in different mobile phase with various ratios of organic phase and buffers by using C18 column. The ideal buffer was 20mm phosphate buffer (pH 6.5) :acetonitrile v/v by isocratic elution to obtain satisfactory and good resolution. The change in pH of mobile phase does not show any significant change in retention time of each analyte. The retention of Cilnidipine and Olmesartan medoxomil on analytical column was evaluated at the flow rate of 1.0 ml/min and injection volume was 20 µl. The retention time of standard and sample for Cilnidipine and olmesartan medoxomil was satisfactory with good resolution.

LINEARITY

The linearity for HPLC method was determined at five concentration levels. The linearity of Cilnidipine and Olmesartan medoxomil were determined by calibration curves and the linearity based on area observed in the range of 10-90 µg/ml for Cilnidipine and 20-180 µg/ml for Olmesartan medoxomil. The relative standard deviation of peak area and the retention time was within the limit. Indicates that, the method were system suitable. The reports are tabulated in **Table-1**.

Table 1: System Suitability Parameters

Parameters	CILNIDIPINE (n=5)	OLMESARTAN MEDOXOMIL (n=5)
Retention Time (min)	4.720	2.655
Peak area	596431	502685
Tailing Factor	0.963	0.000
Theoretical Plates	3445.061	2879.16
Linearity Range	10-90 µg/mL	20-180 µg/mL

Correlation coefficient (R ²)	0.9982	0.9951
Precision (%RSD) Interday	0.932	0.862
Intraday	0.267	0.434
LOD (µg/mL)	0.130	0.790
LOQ (µg/mL)	0.395	2.397

PRECISION

Precision was measured for both inter-day and intra-day and checked with repeatability and the %RSD for the repeatability was found to be 0.267% and 0.434 % respectively for Cilnidipine and Olmesartan medoxomil. The % RSD was found within the limits and tabulated in **Table-1**. The limit of quantification was determined by injecting minimum concentration of drugs. The limit of quantification was found to be 0.39 µg/ml for Cilnidipine and 2.39 µg/ml for Olmesartan medoxomil.

RECOVERY STUDIES

The assay procedure was repeated for standard and sample in five times and mean peak area ratio and concentration of drugs were calculated. The percentage of individual drugs found in formulation, mean, and % RSD in formulation were calculated and show in **Table 2**. Recovery studies carried out for both drugs. It is usually done by adding 80 % , 100 % and 120 % of the pure drug with the formulation taken for analysis. The average % recovery for Cilnidipine and Olmesartan medoxomil was found to be 99.26% and 99.51% respectively. The result was represented in **Table 3**.

Table-2 Analysis of Marketed formulation

Formulation	Cilnidipine			Olmesartan medoxomil		
	Label claim(mg/tab)	Amount found(mg/tab ± RSD)	% assay ± RSD	Label claim(mg/tab)	Amount found(mg/tab ± RSD)	% assay ± RSD
NOXOVAS-O	10	9.90 ± 0.368	99.9 ± 0.896	20	19.8±0.385	98.87±0.875

Table-3 Recovery studies of Cilnidipine and Olmesartan medoxomil in combined dosage form

Level	Cilnidipine		Olmesartan medoxomil	
	% recovered	% recovery ± RSD	% recovered	% recovery ± RSD
80%	17.86	99.29 ± 0.225	35.72	99.14 ± 0.282
100%	19.81	99.05 ± 0.216	39.89	99.76 ± 0.332
120%	21.87	99.46 ± 0.238	43.84	99.64 ± 0.287

SPECIFICITY AND SELECTIVITY

Specificity was tested against standard compounds and potential interferences. To determine specificity with respect to sample compounds the response of standard and sample solution were compared. No interferences were detected at the retention time of either Cilnidipine or Olmesartan medoxomil in sample solution. The limit of detection was determined at lowest concentration giving response and limit of quantification was determined at the lowest concentration. The limit of detection for Cilnidipine and Olmesartan medoxomil was found to be 0.130 µg/ml and 0.790 µg/ml respectively. The limit of quantification was found to be 0.39 µg/ml for Cilnidipine and 2.39 µg/ml for Olmesartan medoxomil and was given in **Table-1**.

RUGGEDNESS AND ROBUSTNESS

Ruggedness test was determined by different analyst in different days using similar operational environmental conditions. Robustness of the method was determined by changing the wave length and flow rate. The content of the drug was not adversely affected by these changes as evident from the low value of relative standards deviation indicating that the method was rugged and robust.

DISCUSSION

Considering the efficiency of HPLC, attempt has been made to develop simple, accurate, precise, rapid and economic method for simultaneous estimation of Cilnidipine and Olmesartan medoxomil in tablet dosage form. Thus method described enables to the quantification Olmesartan medoxomil. Thus advantages lie in the simplicity of sample preparation and the low costs of reagents used. Experimental

results were indicated of satisfactory precision and reproducibility. Hence, this method can be used for analysis of solid dosage form in quality control department. All the analytical validation parameters for these proposed methods were determined according to ICH guideline.⁴

CONCLUSION

The methods described for the determination of Cilnidipine and Olmesartan medoxomil in marketed tablet formulation can successfully employed for the determination of Cilnidipine and Olmesartan medoxomil in marketed formulation for routine analysis in quality control laboratories.

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