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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ANTIHYPERTENSIVE DRUGS OLMESARTAN AND CILNIDIPINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A new reversed-phase HPLC method was developed and subsequently validated for simultaneous estimation of antihypertensive drugs Olmesartan and cilnidipine in pharmaceutical dosage forms. Chromatography was carried out on Inertsil C-18 column (4.6 x 150mm, 5µ particle size) with a mobile phase composed of buffer and acetonitrile in 55:45%v/v and the mobile phase was pumped at a flow rate of 1.0 mL/min. Detection was carried out using a PDA detector at 225nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness were studied as reported in the International Conference on Harmonization guidelines. The retention times for olmesartan and cilnidipine were 2.2 min and 3.7 min respectively. The linearity range for olmesartan and cilnidipine were 10-60 µg/mL and 5-30 µg/mL. The percentage recoveries of olmesartan and cilnidipine were 98.01% and 98.88%, respectively. This method can be employed for routine quality control of olmesartan and cilnidipine tablets in quality control laboratories and pharmaceutical industries.

Key words: RP-HPLC, Olmesartan, Cilnidipine, Simultaneous estimation.

INTRODUCTION

Olmesartan medoxomil, a prodrug, is hydrolyzed to olmesartan during absorption from the gastrointestinal tract. Olmesartan is a selective AT1 subtype angiotensin II receptor antagonist which has been used as antihypertensive drug.

Olmesartan medoxomil is described chemically as 2,3-dihydroxy-2-butenyl 4(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole5-carboxylate, cyclic 2,3-carbonate. It works by blocking the binding of angiotensin II to the AT_1 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[1-2]. Cilnidipine is a unique dihydropyridine derivative Ca2+ channel blocker with an inhibitory action on the sympathetic N-type Ca2+channels. Cilnidipine prevents intracellular calcium influx and results in vasodilatation. Cilnidipine possesses superior selectivity for vascular smooth muscle cells. It is used for hypertension management. Cilnidipine is described chemically as 3-(*E*)-3-Phenyl-2-propenyl 5-2-methoxyethyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate[3-4]. Literature review reveals that few UV, hplc and HPTLC methods are available for the estimation of the simultaneous estimation of Olmesartan and cilnidipine[5-12]. But these methods have some drawbacks. Based on the review, we

planned to develop a new, simple and accurate RP-HPLC method for the simultaneous estimation of Olmesartan and cilnidipine in pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrumentation: Chromatography was performed with Water's 2695 HPLC system provided with Hamilton Syringe, auto sampler and 2996 Photodiode array detector. All HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Data acquisition, analysis, and reporting were performed by Empower2 (Waters) chromatography software.

Reagents and chemicals: Pharmaceutically pure sample of Olmesartan and cilnidipine were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. Acetonitrile and Methanol of HPLC grade was obtained from Merck chemical division, Mumbai and Commercial tablets of Olmesartan and cilnidipine (Nexovas O) were procured from the local drug market.

Chromatographic condition: The mobile phase consisted of buffer: acetonitrile were taken in 55:45%v/v at a flow rate of 1.0 ml/min. Inertsil C-18 column (4.6 x250mm, 5µ particle size) was used as the stationary phase. Although the Olmesartan and cilnidipine have different λ max, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 225 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution: Accurately Weighed and transferred 20mg of Olmesartan and 10 mg of Cilnidipine working Standards into a 100 ml clean dry volumetric flask, add diluent, sonicated for 5 minutes and make up to the final volume with diluents.(standard stock).

Preparation of Working Standard Solutions: Aliquot of 0.5ml, 1.0ml, 1.5 ml, 2.0ml, 2.5ml and 3.0ml were pipetted out from stock into 10 ml volumetric flask separately and volume was made up to 10ml with diluent. This gives the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml & 60 µg/ml for olmesartan and 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml & 30µg/ml for cilnidipine.

Sample preparation: Twenty tablets of Nexovas O containing olmesartan and cilnidipine (20 mg & 10mg, respectively) were weighed and crushed into fine powder. Calculated the average weight of each tablet then the weight equivalent to 5 tablets were transferred into a 250 mL volumetric flask, 160mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Method validation

System suitability tests: To ensure the validity of the analytical procedure, a system suitability test was established. Data from six injections of 10µL of the working standard solutions of olmesartan and cilnidipine were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time.

Linearity: By appropriate aliquots of the standard olmesartan and cilnidipine solutions with the mobile phase, six working solutions ranging between 10-60 µg/mL and 5-30 µg/mL, respectively were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of olmesartan and cilnidipine to obtain the calibration curve.

Accuracy: Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of olmesartan and cilnidipine to which known amounts of standard olmesartan and cilnidipine corresponding to 50%, 100% and 150% of label claim were added. The accuracy expressed as the percentage of analyte recovered by the proposed method.

Precision: Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The repeatability and intermediate precision were determined by analyzing the samples of olmesartan and cilnidipine. Determinations were performed on the same day as well as well as on consequent days.

Limit of detection and the limit of quantification: Limit of detection (LOD) and limit of quantification (LOD) of olmesartan and cilnidipine were determined by calibration curve method. Solutions of both olmesartan and cilnidipine were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations. LOD = (3.3 ×Syx)/b, LOQ= (10.0×Syx)/b

Where Syx is residual variance due to regression; b is slope.

Robustness: The robustness of the method was performed by deliberately changing the chromatographic conditions. The organic strength was varied by ±5%, column temperature was varied by $\pm 5^0$c and the flow rate ±0.1mL.

RESULT AND DISCUSSION

Method development:
Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol: Water, Acetonitrile and Water as mobile phases, in which both the drugs did not responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH: 3.2 both drugs eluted with better separation. Thereafter, buffer: acetonitrile were taken in 55:45%v/v at a flow rate of 1.0 ml/min. Inertsil C-18 column (4.6 x150mm, 5µ particle size) was used as the stationary phase was selected to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 210nm to 280nm. The wavelength at which both Olmesartan and cilnidipine showed maximum absorption at 225nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.2 min and 3.7 min for Olmesartan and cilnidipine, respectively. The obtained chromatogram was shown in the figure 3.

Method Validation:
System suitability: System suitability parameters such as number of theoretical plates, retention time and peak tailing were determined. The results obtained were shown in table-2.

Linearity: Olmesartan and cilnidipine were showed a linearity of response between 10-60 µg/mL and 5-30 µg/mL, respectively (Figure 4 & Figure 5) and the linearity were represented by a linear regression equation as follows.

Y (Olmesartan)=121103.x -12793 (r^2=0.999) Y (cilnidipine)= 141407.x – 6767.6 (r^2=0.999)

Accuracy: The percentage recoveries of olmesartan and cilnidipine were 98.01% and 98.88%, respectively. These results were summarized in table-3.

Repeatability: Six replicates of standard concentrations were analyzed in same day for repeatability and results were found within acceptable limits. These results were summarized in table- 4.

Intermediate Precision: Six replicates of standard concentrations were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits. These results were summarized in table- 5.

Robustness: As per ICH norms, small, but deliberate variations, by altering the Flow rate, column temperature and concentration of the mobile phase were made to check the method's capacity to remain unaffected. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature.

Stability of sample solution: The sample solution injected after 24 hr did not show any appreciable change. Results were shown in table-6.

LOD and LOQ: LOD and LOQ for olmesartan were 0.0146 and 0.0443 µg/mL respectively and for cilnidipine were 0.0017 and 0.0051 µg/mL, respectively.

Tablet Analysis: Content of olmesartan and cilnidipine found in the tablets by the proposed method are shown in Table- 7. The low values of RSD indicate that the method is precise and accurate.

CONCLUSION

RP-HPLC method was developed and validated for simultaneous estimation of olmesartan and cilnidipine in tablet dosage form. The regression value was found to be 0.999 for both olmesartan and cilnidipine , which shows the response is linear from 10-60 µg/mL and 5-30 µg/mL,, respectively. Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of olmesartan and cilnidipine . The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 8 minutes for eluting both Olmesartan and cilnidipine . So, this method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

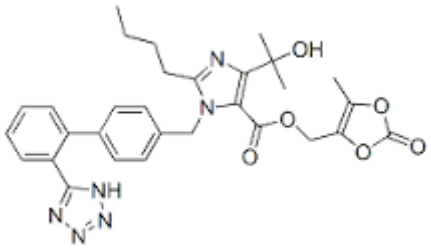

Figure 1: Structure of Olmesartan

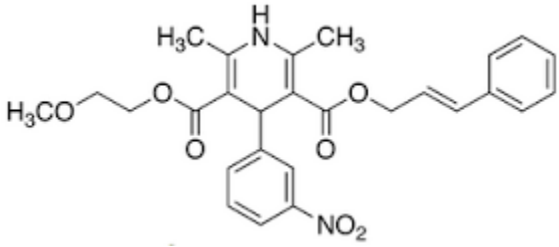

Figure 2: Structure of Cilnidipine

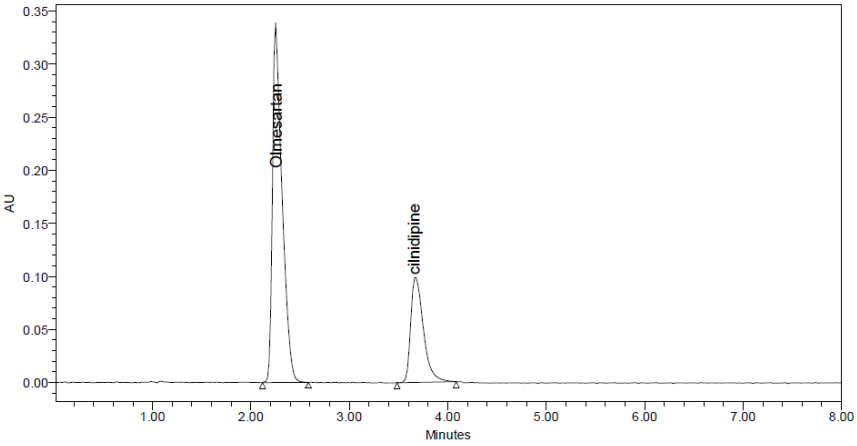

Fig 3: Optimized chromatogram

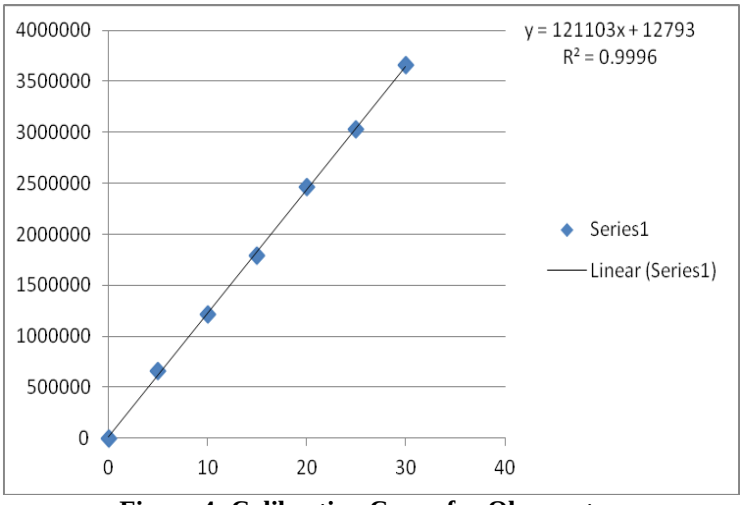

Figure 4: Calibration Curve for Olmesartan

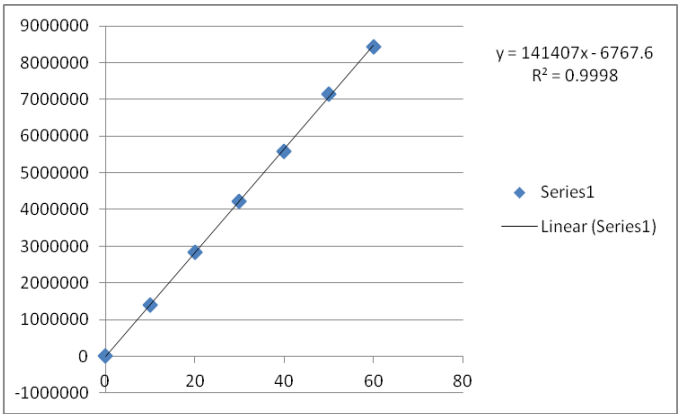

Figure 5: Calibration Curve for Cilnidipine

Table 1: List of previous methods

Ref. No	Existing methods	Drawbacks
5	Column: symmetry C18 (250 x 4.6mm,5 µm Mobile phase: Acetonitrile:Buffer (75:25 %v/v) pH 6.5 adjusted by 1 % Triethylamine	Organic phase concentration is more.
6	Column: Hypersil C18 (250 x 4.6 mm, 5 mm) Mobile phase: Acetonitritle:Phosphate buffer pH 3.6 (70:30 %v/v). Retention time for Olmesartan Medoxomil and Cilnidipine was found to be 4.14 min and 7.79 min respectively	Total runtime is more.
7	Column: HiQSil C18 Mobile phase: Methanol: buffer (90:10v/v) pH adjusted to 3.0	Organic strength in mobile phase is more.
8	Column: C18 (250*4.6*5) Column Mobile phase: mixture of acetonitrile and methanol in the ratio of 60:40	Olmesartan is eluted in Void volume.

Table 2: System suitability of olmesartan and cilnidipine

PARAMETERS	Olmesartan	Cilnidipine
No of theoretical plates	4795	6488
Tailing Factor	1.21	1.34
Retention time	2.2	3.7

Table 3: Results of Recovery Experiments of olmesartan and cilnidipine

Standard drug solution added (ppm)		Spiked Amount (ppm)		% Recovered	
Olmesartan	Cilnidipine	Olmesartan	Cilnidipine	Olmesartan	Cilnidipine
40	20	20	10	99.62	100.24
40	20	20	10	96.44	99.27
40	20	20	10	98.26	98.45
40	20	40	20	99.02	98.68
40	20	40	20	97.68	99.12
40	20	40	20	99.36	98.21
40	20	60	40	98.28	98.29
40	20	60	40	95.84	99.57
40	20	60	40	97.64	98.16
			MEAN	98.01	98.88
			SD	1.27	0.71
			%RSD	1.30	0.72

Table 4: Repeatability data of olmesartan and cilnidipine

S. No.	Olmesartan	Cilnidipine
1	5521475	2456874
2	5614892	2458462
3	5478425	2467841
4	5436524	2461478
5	5478669	2455489
6	5587428	2465472
MEAN	5650821	2460936
SD	17083	4916.064
%RSD	0.30	0.2

Table 5: Intermediate Precision data of olmesartan and cilnidipine

S. No.	Olmesartan	Cilnidipine
1	5498567	2416247
2	5514785	2421458
3	5487596	2458746
4	5521477	2424892
5	5478216	2426587
6	5589686	2436841
MEAN	5515055	2430795
SD	39985.16	15291.756
%RSD	0.73	0.6

Table 6: Stability data of olmesartan and cilnidipine

Drug	%Assay at 0 hr	%Assay at 24hr
olmesartan	99.16	98.42
cilnidipine	98.34	99.01

Table 7: Results of HPLC Analysis of Tablets

Formulation	Label claim (mg)		Amount found (mg)		%Assay	
Nexovas O	Olmesartan	Cilnidipine	Olmesartan	Cilnidipine	Olmesartan	Cilnidipine
	40	20	38.96	19.66	98.15	98.30

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