https://doi.org/10.33472/AFJBS.6.13.2024.1169-1180

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF CILNIDIPINE AND OLMESARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

Dr. Bakhshi Abdurrahman*, Ahire Kiran, Dr. Umair Ahmed, Ambreen Kauser, Dr. Deshmukh Tarique, Dr. Rashid Akhtar

Royal College Of Pharmaceutical Education And Research, Sayne Khurd, Malegaon Dist. Nashik (MS) 423203.

E-mail of Corresponding Author: bakhshiar.jsc@gmail.com

Article Info

Volume 6, Issue 13, July 2024

Received: 02 June 2024

Accepted: 30 June 2024

Published: 24 July 2024

doi: 10.33472/AFJBS.6.13.2024.1169-1180

ABSTRACT:

This study outlines a straightforward, cost-effective, and sensitive stability-indicating reverse phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous quantification of cilnidipine (CNG) and olmesartan (OMS) in bulk and pharmaceutical formulations. The analysis was performed on an octa-decyl C18 column (5 µm, 25 cm x 4.6 mm, i.d) with a mobile phase consisting of 70% acetonitrile (ACN) and 30% 0.1% orthophosphoric acid (OPA) at a flow rate of 1.0 ml/min. Detection was carried out at a wavelength of 257 nm, which was found to be suitable for both cilnidipine and olmesartan. The retention times were 2.78 minutes for cilnidipine and 4.89 minutes for olmesartan. The regression equations for cilnidipine and olmesartan were $y=12352x-7105$ and $y=6634.9x+11282$, with correlation coefficients (R^2) of 0.999 and 0.9989, respectively. This developed method is robust, accurate, and sensitive, making it suitable for the estimation of cilnidipine and olmesartan in combined pharmaceutical dosage forms.

Keywords: Cilnidipine, Olmesartan, RP-HPLC, Simultaneous estimation, Stability Study

1. INTRODUCTION:

Cilnidipine (CNG), (Figure 1) chemically is 3-O-(2-methoxyethyl) 5-O-[(E)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate[1] with a molecular formula of $C_{27}H_{28}N_2O_7$ and a molecular weight of 492.528 g·mol^{-1}. It is White to off-white crystalline powder in colour which is freely soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), and is practically insoluble in water. Cilnidipine decreases blood pressure and is used to treat hypertension and its comorbidities. Due to its blocking action at the N-type and L-type calcium channel, cilnidipine dilates both arteries and veins, reducing the pressure in the capillary bed. Cilnidipine is vaso selective and has a weak direct dromotropic effect, a strong vasodepressor effect, and an arrhythmia-inhibiting effect. Cilnidipine is a medication used primarily for the management of hypertension (high blood pressure). It belongs to a class of drugs known as calcium channel blockers. Cilnidipine works by blocking the influx of calcium ions into smooth muscle cells in blood vessels and the heart, resulting in vasodilation (widening of blood vessels) and reduced peripheral vascular resistance, ultimately lowering blood pressure.
Olmesartan (OMS) chemically described as :(5-methyl-2-oxo-2H-1,3-dioxol-4-yl) methyl 4-(2-hydroxypropan-2-yl) -2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-imidazole-5-carboxylate[2] having empirical formula $C_{29}H_{30}N_6O_6$ and molecular mass 558.595 g·mol^{-1}. It is White to off-white crystalline powder in colour which is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF). Olmesartan functions as an angiotensin-II receptor blocker to undermine the renin-angiotensin-aldosterone system. Olmesartan is an antagonistic molecule that binds to angiotensin type I receptors (AT-I) and angiotensin type II receptors (AT-II).

2. LITERATURE SURVEY:

Literature survey reveals various analytical method validation for Cilnidipine including UV[3-4] HPLC alone or in combination with other drugs including impurity profiling [5-20].

Chemicals and Reagents:
Reference standards of Cilnidipine and Olmesartan were obtained as gift sample from Aristo Pharma, India. Pharmaceutical formulation was purchased from local market (Brand: NEXOVAS O tablet labelled claim OMS 20 mg and CNG 10 mg per tablet make MacLeod's pharmaceuticals).). The HPLC grade solvents used were of E-Merck (India) Ltd., Mumbai. HPLC grade methanol and ortho phosphoric acid (Merck, Mumbai, India) were used in the analysis. HPLC grade water was prepared using Millipore purification system.

Instruments:
Younglin (S.K) gradient system UV Detector with Autochro-3000 database software, RP-C_{18} column (250×4.6 mm), particle size 5µ was used. Sonicator: PCi mumbai, Model No.3.5L 100H.

Chromatographic conditions:
Various combinations of mobile phases were screened with respect to resolution, theoretical plate capacity factors and other system suitability parameters. Finally the separation was performed with freshly prepared mobile phase consist of ACN (70%): 0.1% OPA (30%) with isocratic programming at a flow rate of 1.0 ml/min. 257 nm wavelength, injection volume of 20 µL and ambient temperature was maintained during the entire process to obtain symmetric peaks of CNG and OMS.

Preparation of standard solution:
All solutions were prepared on weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard Pharmaceutical formulation. Standard stock solution was prepared by dissolving separately 10 mg of CNG and 20 mg of OMS in 100 ml clean dry volumetric flask. Dissolved and diluted with methanol up to the mark and filtered through 0.45 µm membrane filter. This gives the concentration of stock solution 200 µg/ml for CNG and 100 µg/ml for OMS.

Linearity study:
From the prepared standard stock solutions of both, 0.5 ml, 1.0, 1.5, 2.0, 2.5 and 3.0 ml were transferred to 10 ml volumetric flask and volume made up to the mark with the optimized mobile phase to obtain concentration of 10-60 µg/ml for CNG, while 20-120 µg/ml for OMS respectively. Volume of 20 µL of each sample was injected with the help of Hamilton Syringe. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area *vs* the drug concentration.

3. VALIDATION OF PROPOSED METHOD:

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Accuracy:
It was done by recovery study using standard addition method at 80%, 100%and 120%level; known amount of standard CNG and OMS were added to pre-analyzed sample (20 µg/ml of CNG; 40 µg/ml of OMS) and subjected them to the proposed HPLC method.
Precision:
Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Intraday and Interday Precision:
Intraday precision were determined by analyzing, the three different concentrations 20 µg/ml, 30 µg/ml and 40 µg/ml of CNG, while 20 µg/ml, 40 µg/ml and 60 µg/ml of OMS for three times in the same day. Day to day variability were assessed using abovementioned three concentrations analyzed on three different days, over a period of one week.

Repeatability:
It is measured by multiple injections of a homogenous sample of 20 µg/ml of CNG and 40 µg/ml of OMS that indicates the performance of the HPLC instrument under chromatographic conditions.

Robustness:
To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate, percentage of methanol using 20 µg/ml solution of CNG and 40 µg/ml of OMS.

Sensitivity:
Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD= 3.3 SD/S and LOQ= 10 SD/S, where SD is the residual standard deviation and S is the slope of the line.

Specificity and selectivity:
The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

Ruggedness:
From stock solutions, sample solutions of CNG (20 µg/ml) and OMS (40 µg/ml) were prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

System suitability test:
System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

Analysis of Pharmaceutical formulation:
To determine the contents of drugs in conventional tablets; Twenty tablets were weighed, their mean weight determined and they were finely powered. Powder equivalent to 20 mg CNG was transferred into a 100 ml volumetric flask containing 50 ml methanol. The resulting solution was sonicated for 30 min and diluted to 100 ml with methanol. The solution was filtered, using 0.45µm filter (Millifilter, Milford, MA). Excipients were separated by filtration. The solution was further diluted with optimised mobile phase to get concentration 10 µg/ml of CNG and 20 µg/ml of OMS which were subjected to proposed method and amount of CNG and OMS were determined.

4. RESULTS AND DISCUSSION:

Optimization of chromatographic conditions:
The primary target in developing this stability indicating HPLC method is to achieve the resolution between Cilnidipine, Olmesartan and its degradation products. To achieve the separation of degradation products, octadecyl silane C_{18} stationary phase and freshly prepared mobile phase consist of ACN: 0.1% OPA in the ratio of 70:30 with isocratic programming at a flow rate of 1.0 ml/min. 257 nm wavelength, injection volume of 20 µL and ambient temperature was maintained during the entire process to obtain symmetric peaks of CNG and OMS. The tailing factor obtained was less than two and retention time was about **2.78** and **4.89** min for OMS and CNG (**Figure 2**). This developed method was found to be specific and method was validated as per international guideline.

Linearity study:
Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Cilnidipine and Olmesartan were found to be as 10-60 µg/ml and 20-120 µg/ml respectively (Table 1). The regression equation for CNG and OMS were found to be as $y = 12352x - 7105$ and $y = 6634.9x + 11282$ with correlation coefficient (R^2) 0.999 and 0.9989, respectively (Figure 3, 4).

Method Validation:

Accuracy:
To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 80%, 100%and 120% concentration levels. Known amounts of

standard CNG and OMS were added to the pre-analyzed samples and were subjected to the proposed HPLC method. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 98.21 to 98.95 % for CNG and 98.49-100.62% for OMS. Results of recovery studies is shown in Table 2.

Precision:
Precision was evaluated by carrying out six independent sample preparations of a single sample by intra-day and inter-day precision. The sample preparation was carried out in same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is precise shown in Table 3.

Repeatability:
It is measured by multiple injections of a homogenous sample of 200 µg/ml of CNG and 40 µg/ml of OMS and the % R. S. D. was found to be less than 2 (Table 4).

Robustness of the method:
To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were done. The effects of change in flow rate, retention time, and in mobile phase ratio were studied. The method was found to be unaffected by small changes like +/- 10% in flow rate, +/- 0.2 change in pH, shown in Table 5.

Sensitivity:
LOD and LOQ can be determined based on visual evaluation, signal-to-noise approach and standard deviation of the response and slope. Limit of detection of CNG and OMS was determined 0.613 and 1.005, respectively. Limit of quantitation of CNG and OMS was determined 1.857 and 3.045, respectively.

Specificity and selectivity:
The method is quite selective. There were no other interfering peak around the retention time of CNG and OMS; also the base line did not show any significant noise.

Ruggedness:
Different analyst carried out precision studies in a similar manner carried out by first analyst. The % Assay was found to be 99.40-99.58%, and 99.60-99.80% of CNG and OMS, respectively. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is rugged, shown in Table 6.

System suitability test:
System suitability testing is essential for the assurance of the quality performance of the chromatographic system. The tailing factor, capacity factor, and theoretical plates for CNG and OMS were in the acceptance criteria as per the ICH guidelines (Table 7).

Analysis of Pharmaceutical formulation:
The assay procedure was repeated for six times; the percentage content of CNG and OMS in the tablet formulation was determined as 99.434-100.268 % and 98.6-101.25% respectively (Table 8).

Procedure for Forced Degradation Study:
Forced degradation of each drug substances and the drug product was carried out under acidic, basic, oxidative stress, thermolytic and photolytic, conditions. Thermal degradation of

drug was carried out in solid state. While remaining all studies were carried out in solution form. Solutions were prepared by dissolving drug with distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide, or aqueous hydrogen peroxide solution, which is further diluted with mobile phase to achieve a concentration of 20 µg/ml each of CNG and 40 µg/ml for OMS. These solutions were kept for 1 Hr. For thermal stress, samples of drug was placed in a controlled-temperature oven at 50ºC for 1 hr. Solutions of drug substances and drug product were also kept at 80 ◦C for 48 h. For photolytic stress, samples of drug in solution state, was irradiated with UV radiation having peak intensity at 254 and 366 nm. The degradation studies (Figure 4-7) were tabulated in Table 09.

5. CONCLUSION:

The present study was conducted to develop and validate a simple, sensitive and reproducible RP-HPLC method for quantitative determination of Cilnidipine and Olmesartan with stressed stability studies under different conditions. The developed chromatographic assay fulfilled all the requirements to be identified as simple, specific, selective and reliable method, including accuracy, linearity, recovery and precision data.
Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of Cilnidipine and Olmesartan in pharmaceutical formulations without any interference from the Excipients and degraded peaks.

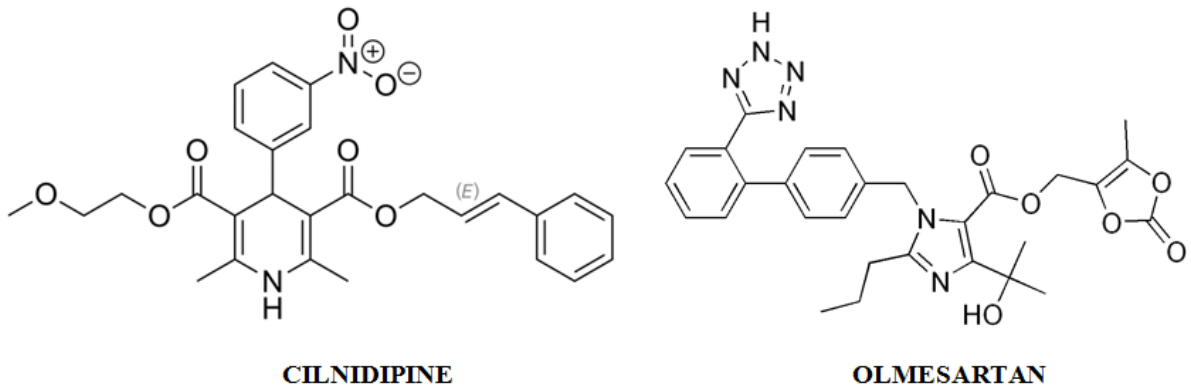


Figure 1: Chemical Structures of CNG [A] AND OMS [B]

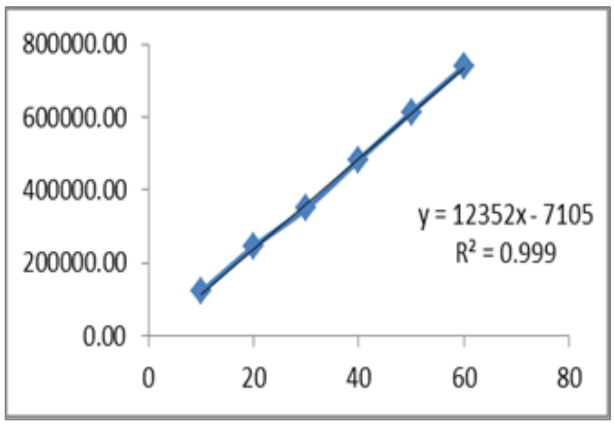
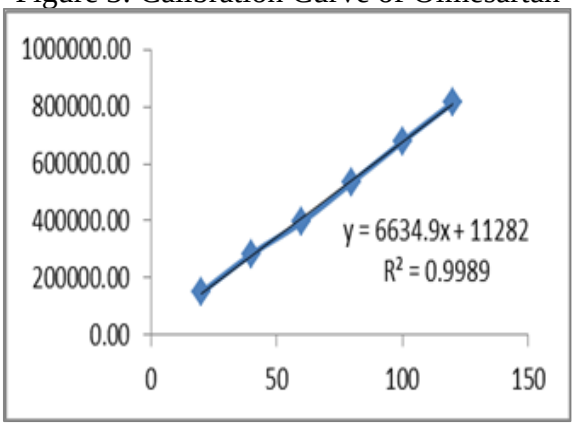


Figure 2: Calibration Curve of Cilnidipine

Figure 3: Calibration Curve of Olmesartan



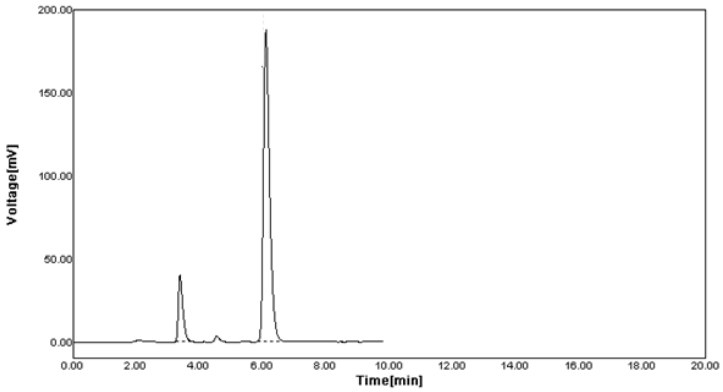


Figure 4: Chromatogram of Standard Cilnidipine Hydrochloride and Olmesartan at 257 nm

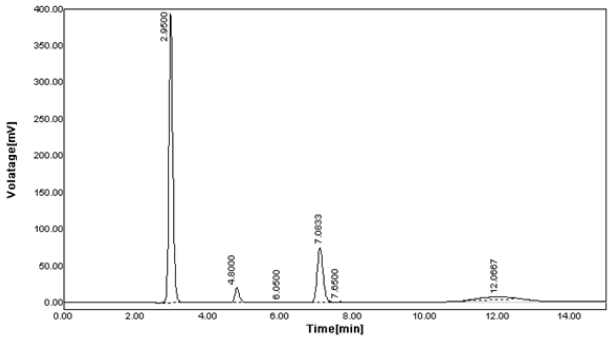


Figure 5: Acidic Degradation (1N, HCl) After 1 Hr

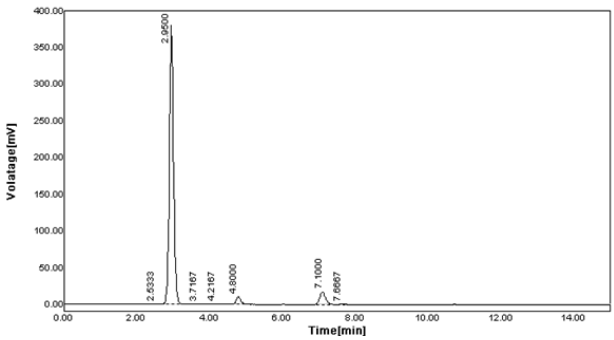


Figure 6: Alkaline Degradation (1 N NaOH) After 1 Hr

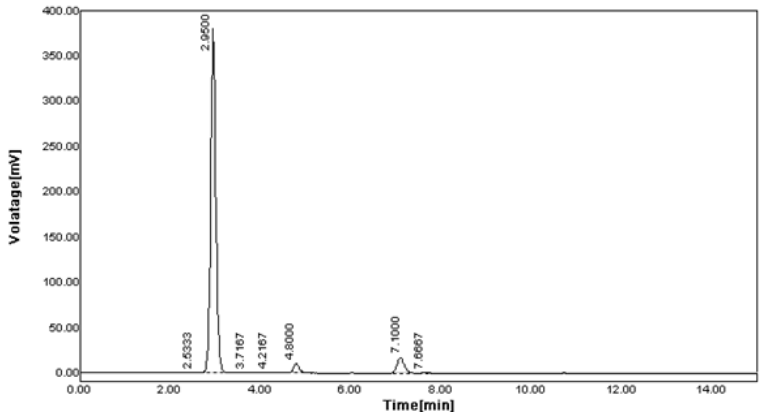


Figure 7: Per Oxide Degradation (30% H_2O_2) After 1 Hr

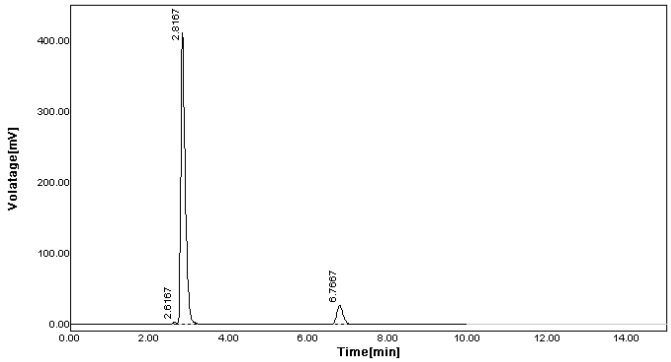


Figure 8: Heat Degradation at 50^{o}c For 1 Hr

Table 1: Linearity Study of CNG And OMS

Sr. No.	CNG				OMS			
	Conc [µg/ml]	Mean peak area [n=5]	± SD	%RSD	Conc [µg/ml]	Mean peak area [n=5]	± SD	%RSD
01	10	121259.20	2202.87	1.82	20	148727.20	1984.68	1.33
02	20	245132.00	2019.01	0.82	40	283311.20	2876.02	1.02
03	30	350392.60	6386.26	1.82	60	395969.60	4294.92	1.08
04	40	482820.60	7020.53	1.45	80	535764.00	8126.13	1.52
05	50	613122.20	9168.62	1.50	100	677329.00	5273.58	0.78
06	60	738647.60	5553.55	0.75	120	813242.20	5358.22	0.66

Table 2: Results of Recovery Studies of CNG and OMS

Drug	Initial Amt [µg/ml]	Amt added [µg/ml]	Amt recovered ± S.D. [µg/ml, n = 3]	% Recovery	% RSD
CNG	20	0	20.32± 0.67	102.87	0.89
	20	16	20.48± 0.89	101.46	1.49
	20	20	20.43± 1.09	100.40	1.45
	20	24	20.38± 1.28	102.46	1.42
OMS	40	0	41.15± 0.27	102.78	1.83
	40	32	40.16± 0.20	102.87	1.67
	40	40	40.98± 0.24	101.46	1.58
	40	48	41.11± 0.18	100.40	1.02

Table 3: Results Of Precision Studies of CNG And OMS (Intra-Day And Inter-Day)

Drug	Conc. [µg/ml]	Intra day Amt Found [µg/ml]		Inter day Amt Found [µg/ml]	
		Mean ± SD	% RSD [n= 3]	Mean ± SD	% RSD [n= 3]
CNG	20	20.44± 4.16	**0.11**	20.23± 8.50	**0.07**
	30	30.96± 10.21	**0.74**	29.53± 7.64	**0.50**
	40	39.80± 6.66	**0.12**	99.50 ± 9.45	**0.16**
OMS	40	39.71± 2.00	**0.24**	39.73± 2.00	**0.14**
	60	61.89± 3.06	**0.24**	61.90± 5.57	**0.14**
	80	76.05±5.51	**0.26**	75.60± 5.03	**0.25**

Table 4: Results of Repeatability Study of CNG And OMS

CNG			OMS	
Sr. No.	Concentration [µg/ml]	Peak area	Concentration [µg/ml]	Peak area
1	20	132052	40	457118
2	20	133143	40	450974
3	20	132805	40	446318
4	20	132805	40	455346
5	20	132805	40	456733
6	20	132429	40	453298
Mean ± SD		**132673.33± 379.00**	Mean ± SD	**453297.92± 4115.82**
% RSD		**0.29**	% RSD	**0.91**

Table 5: Robustness Evaluation of the HPLC Method

Chromatographic conditions (CNG)	T Tailing	K' Capacity Factor	N Theoretical Plate
A: Flow rate (ml/min.)			
0.90	1.32	1.09	246941
1.0	1.18	1.04	245689
1.1	1.23	1.21	245691
Mean ± SD	1.18 ± 0.03	1.12 ± 0.08	246107 ± 722.2659
B: Percentage ACN in mobile phase (v/v)			
60	1.10	1.22	245691
70	0.98	1.13	245686
80	1.21	1.18	245132
Mean ± SD	1.09 ± 0.11	1.17 ± 0.04	245503 ± 321.3052
A: Flow rate (ml/min.)			
0.90	1.18	1.03	285699
1.0	1.14	1.16	281859
1.1	1.09	1.08	278949
Mean ± SD	1.13 ± 0.04	1.09 ± 0.04	282169 ± 3385.661
B: Percentage ACN in mobile phase (v/v)			
60	1.14	1.21	278949
70	1.42	1.12	284591
80	1.23	1.19	285458

Mean ± SD	1.26 ± 0.14	1.17 ± 0.04	282999.3 ± 3534.377

Table 7: System Suitability Test For CNG And OMS

System suitability parameters	Proposed method for CNG	Proposed method for OMS
Retention time (Rt)	2.78	4.89
Capacity factor (K')	1.35	1.30
Theoretical plate (N)	**3698**	**5956**
Tailing factor (T)	1.08	1.16

Table 8: Analysis of Tablet Formulation

Drug	Label claim [mg]	Amount found [mg]	Amount found [%]±SD	%RSD
CNG	10	10.05	100.5 ± 0.28	0.78
OMS	20	20.06	100.3± 0.48	1.28

Table: 09: Forced Degradation of CNG and OMS

Sample Exposure condition	Total Number of products with their Rt	OMS		CNG	
		Degradation remained (40 µg/ml)	Recovery (%)	Degradation remained (20 µg/ml)	Recovery (%)
Acidic, 1N, 1 h	6 (2.95, 4.80, 6.05, 7.08, 7.65, 12.06)	36.22	91	17.56	87.8
Basic, 1N, 1 h	6 (2.61, 2.80, 2.95, 3.38, 4.51, 7.20)	30.96	77	16.89	84.5
Per oxide, 30 %, 1 h	4 (2.63, 2.83, 4.76, 7.03)	37.58	94	18.15	90.8
Heat, 50 °C, 1 h	3 (2.61,2.81,6.766)	39.65	99	19.45	97.3

Acknowledgments:
The authors show sincere thanks to Hetre Pharmaceuticals, Hyderabad for providing the drug samples. Special and sincere thanks to the staff of the Royal College of Pharmaceutical Education and Research (RCPER) for permitting us to carry out the research work.

6. REFERENCES:

1. https://go.drugbank.com/drugs/DB09232
2. https://go.drugbank.com/drugs/DB00275
3. Safwan M Fraihat and Hatim S Khatib. Development and validation of spectrophotometric and spectrofluorimetric methods for determination of cilnidipine; Tropical Journal of Pharmaceutical Research. 2020, 19 (7):1503-1509.
4. Chakravarthi KSS, Ilango KB, Kishore Kumar U, Mirudula G and Saranya S. Development and Validation of UV-Spectroscopic Method for the Estimation of Cilnidipine in Bulk and Pharmaceutical Dosage Form; world journal of pharmaceutical and medical research. 2023, 9(4):119-123.

5. Bapi Gorain, Hira Choudhury, Easha Biswas, Anwesha Barik, P. Jaisankarb and Tapan Kumar Pal. A novel approach for nanoemulsion components screening and nanoemulsion assay of olmesartan medoxomil through a developed and validated HPLC Method; RSC Advances. 2013, 3:10887-10894.
6. Tiwari Balakrishna, Shirsat Mrunal K and Kulkarni Amol. Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Cilnidipine; Journal of Drug Delivery and Therapeutics. 2020, 10(1):97-100.
7. Sunitha N, Subash C M, Sai Sravanthi J, Venu A, Narasimha Rao BV and Appa Rao B. Method Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Olmesartan and Cilnidipine in Bulk and Formulations; International Journal of Pharmaceutical Research & Allied Sciences. 2015, 4(3):127-135.
8. Ravi Sankar P, Swathi V and Srinivasa Babu. Development and Validation of Novel UV and RP-HPLC Methods for Determination of Cilnidipine (A New Generation Ca Channel Blocker) In Pharmaceutical Dosage Form: *International Journal of Pharmaceutical Sciences and Research.* 2019, 10(4): 1886-1894.
9. Radhika Sojitra and Urvi Chotaliya. Analytical method development and validation for simultaneous estimation of Fimasartan Potassium Trihydrate and Cilnidipine in synthetic mixture by HPLC for the treatment of hypertension stage-II; Future Journal of Pharmaceutical Sciences. 2021, 7:189-196.
10. Manoranjani M. assay method development and validation of cilnidipine and ramipril, characterization of its degradants by using LC-MS/MS; International journal of Applied Pharmaceutics. 2022, 14(2):276-285.
11. Reema H Rupareliya and Hitendra S. Joshi Stability Indicating Simultaneous Validation of Telmisartan and Cilnidipine with Forced Degradation Behavior Study by RP-HPLC in Tablet Dosage Form; ISRN Chromatography. 2013, 461461:1-6.
12. Arvind Kumar, Surya Prakash Dwivedi and Tulika Prasad. Method Validation for Simultaneous Quantification of Olmesartan and Hydrochlorothiazide in Human Plasma Using LC-MS/MS and Its Application through Bioequivalence Study in Healthy Volunteers; Frontiers in Pharmacology. 2019, 10:810-823.
13. Chimalakonda Kameswara Rao, Kakumani Kishore Kumar, Maddala VijayaLaxmi, Polisetty Srinivasulu, Gutta Madhusudhan, Khagga Mukkanti and Koduri Sai Venkata Srinivas. Development and Validation of Stability Indicating LC Method for Olmesartan Medoxomil; American Journal of Analytical Chemistry. 2012, 3:153-160.
14. Pritesh R Desai1, Priti J Mehta and Avani B Chokshi. Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Quantification of 15 Organic Impurities of Olmesartan Medoxomil, Amlodipine and Hydrochlorothiazide in Combined Dosage Form; Chromatographia. 2019, 82:819–833.
15. Ritesh N Sharma and Shyam S Pancholi. RP-HPLC-DAD method for determination of olmesartan medoxomil in bulk and tablets exposed to forced conditions; Acta Pharmaceutica.2010, 60:13–24.
16. Mithun Rudrapal, Madhavi Usharani Oduri, Nageswara Rao Samidala, Surya Kiran B, Julfikar Ali Junej, Khumantham Deepak Singh, Tapash Chakraborty and Manabendra Debnath. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Olmesartan and Hydrochlorothiazide in Tablet Dosage Form; Oriental Journal of Chemistry. 2015,31(2):921-926.
17. Rathod Dipak, Dubey Archana and Chaturvedi Prerna. Method development and validation of olmesartan and hydrochlorothiazide by UV spectroscopy; International Journal of Pharmaceutical Sciences and Medicine. 2021, 6(8):17-31.
18. Vilas Sawale, Pankaj Dangre and Disha Dhabarde. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Olmesartan Medoxomil and

Chlorthalidone in Tablet Dosage Form; International Journal of Pharmacy and Pharmaceutical Sciences. 2015, 7(5),266-269.

19. Nitin Dubey, Nidhi Dubey, Mayank Mandhanya and Dinesh Kumar Jain. Cleaning validation for residual estimation of olmesartan medoxomil on stainless steel surface of pharmaceutical manufacturing equipment's using swab sampling and HPLC-DAD method; Bulletin of Faculty of Pharmacy. 2013, 51:95–100.
20. Srinivas A and Sneha Y. Stability Indicating Forced Degradation RP-HPLC Method Development and Validation of Olmesartan Medoxomil; International Journal of Pharmaceutical Sciences and Research. 2014, 5(7): 2848-2855.