



Method Development and Validation of RP-HPLC Method for determination of Clinidipine and Telmisartan in Bulk and Pharmaceutical formulation

¹Dr.Ganesh Akula, ²N.Mamatha, ²M.Kishor, ²P.Sannith, ²B.Santhosha

¹Associate Professor, ² Student

Department of Pharmaceutical Chemistry

Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda, Gajwel, Siddipet, Telangana.

ABSTRACT:

In order to measure Telmisartan and Clinidipine concurrently in pharmaceutical formulations and bulk, a Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method was created and verified. The process used a Phenomenex Gemini C18 column (4.6 × 150 mm, 5.0 µm) and a mobile phase consisting of methanol and water in a 32:68% (v/v) ratio. A wavelength of 248 nm, a flow rate of 1 ml/min, an injection volume of 20 µl, a column temperature of 38°C, and a run time of 10 minutes were all part of the analytical parameters. Effective separation was ensured by optimizing the method to produce distinct, sharp peaks for every medication.

The method's robustness and dependability were verified, and it met all the requirements for specificity, linearity, precision, accuracy, and sensitivity. The findings showed that the technique, which yields accurate telmisartan and clinidipine measurements, is appropriate for regular quality control and assurance in the pharmaceutical sector. For both bulk and prepared items, the RP-HPLC technology is a useful tool for guaranteeing the proper dosage and effectiveness of these antihypertensive drugs.

Key words: Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC), Clinidipine, Telmisartan, Method Development, Method Validation, Pharmaceutical Formulations.

INTRODUCTION

To learn more about the composition and characteristics of matter, the scientific discipline of analytical chemistry develops and uses instruments, methods, and processes. Analytical chemists or quality control chemists are in charge of creating sensitive, trustworthy, and precise methods for assessing the drug in pharmaceutical dosage forms and biological fluids. The current developments in the pharmaceutical sector are mostly the result of analytical chemistry.

High-performance liquid chromatography (HPLC) is an analytical technique that uses different rates of elution to resolve solutes as they pass down a chromatographic column. This device uses a separation process that is controlled by the distribution between the stationary phase and mobile phase. Reversed-phase chromatography is currently the most popular separation technique in HPLC because of its many uses. It is estimated that over 65% (and most likely up to 90%) of all HPLC separations are performed in the reversed phase mode. This is due in part to the reversed-phase method's versatility, reach, and convenience of use, which can handle compounds with a broad range of polarity and molecular mass¹⁻³. The methods validation process for analytical procedures⁴ begins when the applicant collects the validation data to support analytical procedures in a systematic and organized manner.

The ability of a column to retain sample and successfully separate sample components during an isocratic separation is indicated by the capacity factor (k'), which is the ratio of the reduced retention volume to the dead volume. For k' , the ideal range is 2–10. The capacity factor can be computed using the formula below:

$$K' = \frac{V_r - V_o}{V_o}$$

Where, V_r = retention volume at the apex of the peak (solute) and V_o = Void volume of the system⁵.

Angiotensin II receptor blockers (ARBs), such as telmisartan (TEL), reduce the effects of angiotensin II-mediated aldosterone synthesis by selectively blocking the subtype 1 (AT1) receptor. As a result, blood pressure is lowered because there is a decrease in the excretion of water and sodium and an increase in the excretion of potassium. TEL is chemically 5-(4-(1-Hydroxy-1-methylethyl) phenyl) benzimidazol-2-yl) methyl-2-(2-carboxyphenyl) thiazole-4-carboxylate, with the molecular formula $C_{33}H_{30}N_4O_2$ and a molecular mass of $514.629 \text{ g}\cdot\text{mol}^{-1}$. TEL is soluble in organic solvents like ethanol and methanol and has a 50% bioavailability; however, it is nearly insoluble in water. In high-risk patients, it is primarily used to treat hypertension and reduce the risk of cardiovascular events. Telmisartan should not be used by those who are pregnant, have severe renal impairment, or are hypersensitive to it⁶.

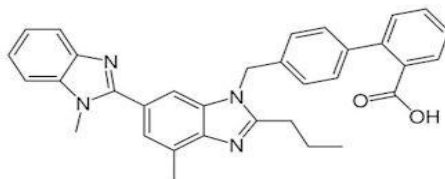


Figure 1: Structure of Telmisartan

Clinidipine, a calcium channel blocker (dihydropyridine derivative), lowers blood pressure and promotes vasodilation by blocking L-type calcium channels that allow calcium to enter cardiac and vascular smooth muscle. Thiazol-2-yl 2-(4-(2-(2, 2-dichloroethoxy) phenyl) Its chemical name is -5-phenyl-1, 4-dihydro-2, 6-dimethylpyridine-3,5-dicarboxylate. Its molecular weight is $492.528 \text{ g}\cdot\text{mol}^{-1}$, and its chemical formula is $C_{27}H_{28}N_2O_7$. CYP_{3A4} in the liver metabolizes it extensively, used to treat hypertension⁷ and chronic stable angina.

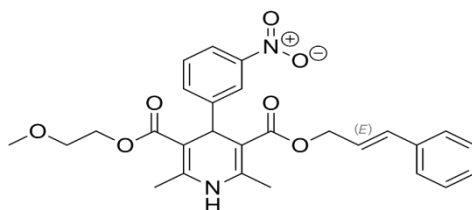


Figure 2: Structure of Clinidipine

According to the literature review, telmisartan and clinidipine, either separately or in combination with other formulations, can be measured using the spectrophotometric,^{8,9,10} RP-HPLC method^{11,12,13}. The Indian¹⁴, British, and Japanese pharmacopoeias all recognize telmisartan as an official medication.¹⁵

As part of this study, a reliable, accurate, and efficient RP-HPLC method for the simultaneous detection of Clinidipine and Telmisartan in pharmaceutical formulations and bulk drug substances is being developed and validated.

MATERIALS AND METHODS:

Materials and Reagents:

Telmisartan and Clinidipine samples were obtained from Sun Parma Pvt. Ltd. and provided by Sura Pharma Labs in Hyderabad. Every solution was inspected and protected from the light on the day of preparation. All of them were of the analytical-reagent (AR) variety. Along with Millipore water and HPLC-grade methanol from LICHROSOLV (MERCK), acetonitrile was acquired from Merck, India. They used glassware made of borosil.

Instrumentation:

With a wavelength of 248 nm, the PDA Detector 996 model and the WATERS Alliance 2695 separation module with auto sampler were employed for the high performance liquid chromatography (HPLC) analysis using reversed-phase columns. The data was recorded and analyzed using Empower 2. A Lab India pH meter was used to measure the pH after Sartorius was weighed.

Chromatographic Conditions:

The chromatographic analysis was performed using the Phenomenex Gemini C18 (4.6 x 150 mm, 5.0 μm) as the instrument. The mobile phase is made up of methanol and phosphate water in a 32:68 v/v ratio and is pumped at a rate of 1 milliliter per minute. At 248 nm, the eluents were identified using a 20 μL injection volume. 38°C was the temperature at which this entire procedure took place.

Preparation of standard solution:

Put 10 mg of the working standards for telmisartan and clinidipine into a 10 ml volumetric flask that has been thoroughly cleaned and dried. To get the volume up to the appropriate level, add around 7 ml of methanol, sonicate to completely dissolve the air, and then use the same methanol. Furthermore, pipette 2.25 ml of the previously mentioned Clinidipine and 0.45 ml of the Telmisartan stock solutions into a 10 ml volumetric flask. Dilute with methanol to the proper concentration.

Mobile phase preparation: Preparing the mobile phase: precise measurements After mixing and degassing 320 milliliters (32 percent) of HPLC methanol and 680 milliliters (68 percent) of water in a digital ultra sonicator for fifteen minutes, the mixture was vacuum-filtered through a 0.45 μm filter. The diluents were the mobile phase..

Preparing the Exemplar Solution:

Using a pestle and mortar, crush the tablet to its average weight. Next, add 10 milligrams of Telmisartan and an equivalent weight of Clinidipine to a 10-mL volumetric flask that has been cleaned and dried. After adding roughly 7 mL of diluents and sonicating them to completely dissolve them, use the same solvent to increase the volume to the necessary level. Additionally, pipette 0.3 ml of the Telmisartan stock solutions and 0.5 ml of the previously mentioned Clinidipine stock solutions into a 10 ml volumetric flask. Adjust the level by diluting with diluents.

Method Validation:

As per the ICH guidelines the method validation was done for the parameters like system suitability, stability, and the validation

RESULTS AND DISCUSSION

Optimized chromatographic condition:

According to preliminary investigations, the peaks of Telmisartan and Clinidipine were separated by using various methanol and water combinations, which resulted in appropriate retention durations and peak symmetry. Good resolution and adequate peak symmetry were obtained using Phenomenex Gemini C18 (4.6×150mm, 5.0 µm) and a mobile phase composition of methanol and water in a 32:68 v/v ratio. We experimented with flow rates ranging from 1.0 to 1.2 ml/min. It was found that a flow rate of 1.0 ml/min was sufficient to elute both medications in less than ten minutes. Telmisartan and Cilnidipine's UV spectra show a good absorption at 248 nm. The optimized chromatogram of standard and sample were shown in Fig.3 and Fig.4 respectively

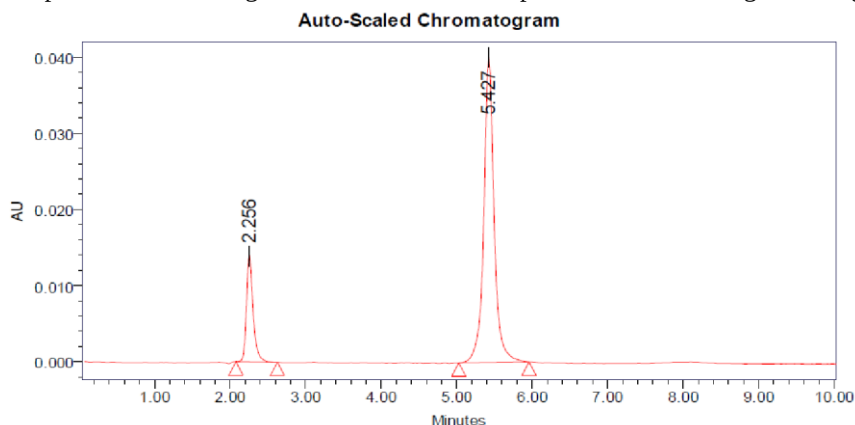


Figure 3: Optimized Chromatogram (Standard)

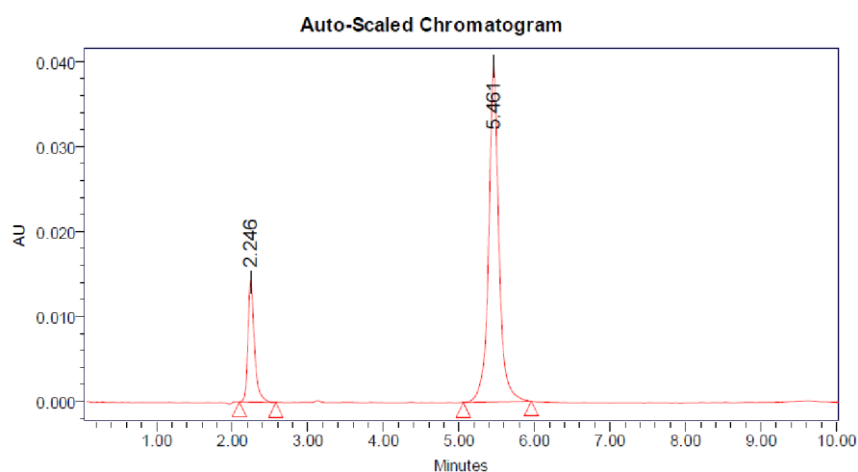


Figure 4: Optimized Chromatogram (Sample)

Method Validation:

Specificity: The peak purity of Telmisartan and Cilnidipine during the force degradation testing was used to evaluate the proposed method, and the interference of a placebo with the analyte was used to determine the procedure's specificity. Telmisartan and Cilnidipine demonstrated good peak purity levels of 0.999 and 0.999, respectively, under a range of stress conditions. The drug peak was unaffected by any degradation product peak. Tables Nos. 1 and 2 displayed the specificity results.

Table1: Peak results for assay standard

S. No	Drug Name	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Telmisartan	5.439	371860	39230	16.10	1.06	9180
2	Clinidipine	2.244	85430	13980	--	1.37	3625
3	Telmisartan	5.421	378990	40110	16.22	1.04	9270
4	Clinidipine	2.251	86580	14100	--	1.39	3710
5	Clinidipine	2.246	86100	14020	--	1.35	3645
6	Telmisartan	5.429	375120	39540	16.18	1.02	9025

Table2: Peak results for assay sample

S. No	Drug Name	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Clinidipine	2.251	85380	35800	--	1.35	9450
2	Telmisartan	5.458	372600	38420	16.38	1.36	9475
3	Clinidipine	2.248	86410	33420	--	1.34	9442
4	Telmisartan	5.465	368210	39620	16.37	1.05	9190
5	Clinidipine	2.246	83890	39250	--	1.04	9280
6	Telmisartan	5.471	381900	39070	16.43	1.03	9185

Linearity: A linearity test solution for the assay technology was created using five levels of concentrations (30, 40, 50, 60, 70 mcg/ml) of assay analytical concentrations. The correlation coefficient for telmisartan was 0.999, which was for Clinidipine 10, 20, 30, 40, and 50 mcg/mL. Peak regions for concentration data were assessed using linear regression analysis. Linearity data (Table 3) and calibration curves (Figures 5 and 6) show the accuracy and reliability of the method.

Table 3: Linearity data for Cilnidipine and Telmisartan

Clinidipine		Telmisartan	
Concentration [g/ml]	Average Peak area	Concentration [g/ml]	Average Peak area
30	50250	10	221200
40	91000	20	437800
50	137800	30	630500
60	179500	40	837000
70	221300	50	1035000

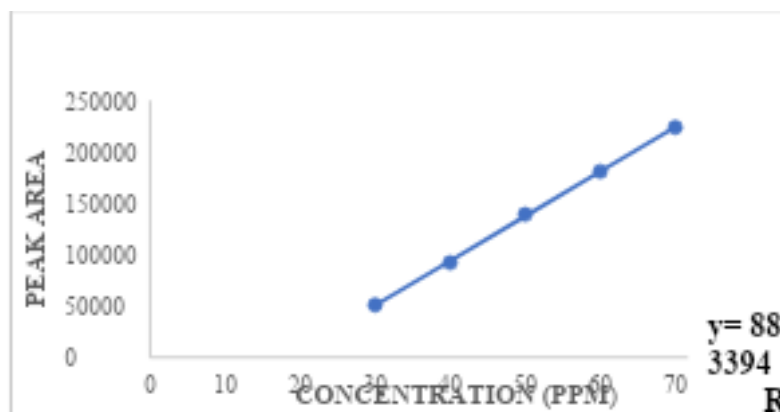


Figure5: Calibration graph for Clinidipine

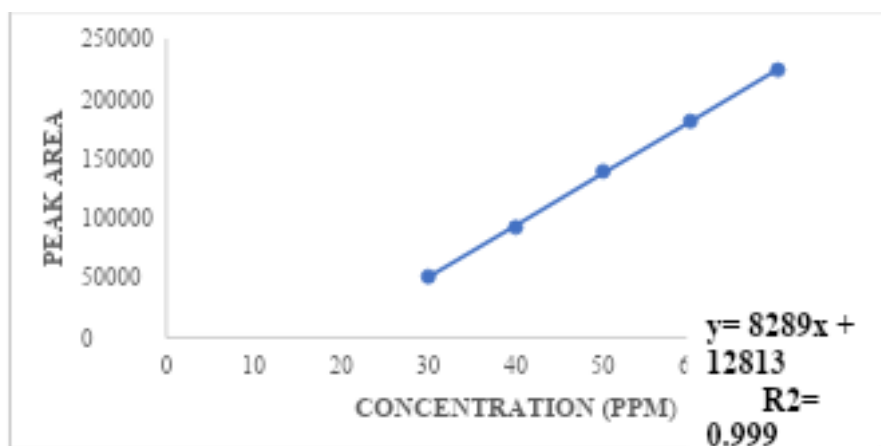


Figure 6: Calibration graph for Telmisartan

LOD and LOQ: Serial dilutions of telmisartan and silnidipine stock solutions were used to assess detection limits of 2.4 µg/mL or 0.54 µg/mL or quantitative limits.

Precision: Three separate tests were performed, telmisartan and sirnidipin test sample samples, and the accuracy of the assay was assessed using reproducibility by calculating the RSD percentage (intraday) of the assay. The intermediate precision of the method was verified by running the same process under experimental conditions on different days (interlay). Accuracy findings were presented in Tables 4 and 5

Table 4: Day-1 Results of intermediate precision for Clinidipine

S. No	Drug Name	Rt	Area	Height	USP platecount	USP Tailing
1	Clinidipine	2.248	84029	13603	3519.3	1.4
2	Clinidipine	2.245	84202	13520	3372.9	1.4
3	Clinidipine	2.242	84745	13636	3411.8	1.4
4	Clinidipine	2.239	85442	13775	3323.5	1.3
5	Clinidipine	2.243	85535	13768	3433.4	1.4
6	Clinidipine	2.246	85699	13739	3336.9	1.3
Mean			84942			
Std. Dev			720.3716			
% RSD			0.8			

Table 5: Day-1 Results of intermediate precision for Telmisartan

S. No	Drug Name	Rt	Area	Height	USP platecount	USP Tailing	USP Resolution
1	Telmisartan	5.284	366831	40102	9180.2	1.1	15.8
2	Telmisartan	5.293	368856	40464	9155.6	1.1	15.5
3	Telmisartan	5.306	370174	39977	9039.6	1.0	15.5
4	Telmisartan	5.319	370603	40748	9119.3	1.1	15.8
5	Telmisartan	5.346	372578	39772	9183.9	1.1	15.6
6	Telmisartan	5.352	376550	40083	9009.1	1.1	15.9
Mean			370932				
Std. Dev			3349.09				
% RSD			0.9				

Accuracy: sourced and real concentrations. Three concentration levels (equivalent to 50, 100, and 150% of the test preparation concentration) were used to assess the method's recovery. Three sets were made and injected in duplicate for every concentration level. Results for accuracy were displayed in Tables Nos. 6 and 7.

Table 6: Accuracy results for Clinidipine

% Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	69862.33	7.5	7.47	99.6	98.8%
100%	135467.7	15	14.8	98.6	
150%	199976	22.5	22.1	98.2	

Table 7: Accuracy results for Telmisartan

% Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	322954	37.5	37.4	99.7	99.7%
100%	632155	75	74.7	99.6	
150%	945870.3	112.5	112.5	100	

Robustness: To assess the impact of minor but intentional changes on the chromatographic conditions, a robustness study was conducted. The flow rate (± 0.1 mL/min) was one of the criteria selected for this investigation. Tables Nos. 8 and 9 displayed the robustness results.

Table 8: Results for Robustness of Clinidipine

Parameters	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flowrate of 1.0 mL/min	84994	2.256	5535	1.32
Less Flowrate of 0.9 mL/min	89987	2.505	5891	1.27
More Flowrate of 1.1 mL/min	80653	2.046	5085	1.20
Less organic phase	89987	2.505	5098	1.20
More organic phase	80654	2.046	5123	1.27

Table 9: Results for Robustness of Telmisartan

Parameters	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flowrate of 1.0 mL/min	377906	5.427	9101	1.01
Less Flowrate of 0.9 mL/min	397680	5.599	9407	1.03
More Flowrate of 1.1 mL/min	327899	4.576	9584	0.98
Less organic phase	396750	5.599	9407	1.02
More organic phase	339025	4.576	9584	0.99

CONCLUSION:

The RP-HPLC technique was created to make sure that Telmisartan and Clinidipine were both sufficiently resolved with distinct, sharp peaks and that the method was trustworthy and strong enough to be used in pharmaceutical formulations as well as bulk. The active pharmaceutical components are effectively separated and quantified using the optimized method parameters, which include a Phenomenex Gemini C18 column, a mobile phase of methanol and water (32:68% v/v), and the designated analytical conditions. The approach satisfies all required requirements for specificity, linearity, precision, accuracy, and sensitivity, according to the validation results. Clinidipine and Telmisartan's correct dosage and effectiveness in their individual formulations will be guaranteed by this technique, which will be a useful tool for quality control and assurance in the pharmaceutical sector.

ACKNOWLEDGEMENT:

For providing the facilities I needed to conduct my research, the authors are grateful to Smt. Surabhi Vani Devi, Chairman of the Surabhi Educational Society; Dr.Venkataramana, Principal of the Surabhi Dayakar Rao College of Pharmacy; and Sura Rajini, Managing Director, Sura Pharma Labs Hyderabad.

CONFLICT OF INTEREST:

Regarding this paper publication, the authors state that they have no conflicts of interest.

REFERENCES:

1. Willard HH and Dean AJ. Instrumental Methods of Analysis. CBS Publishers and distributors, 7th ed, 1986, 513-515.
2. Connors AK. In: A Text Book of Pharmaceutical Analysis. A Wiley Interscience Publication, 3rd ed, 2005, 373-400.
3. Ahuja S. In: High Pressure Liquid Chromatography of Comprehensive Analytical Chemistry. Elsevier Publications. 2006.
4. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.
5. Neue UD. HPLC Columns: Theory, Technology and Practice. 2nd Ed. New York: John Wiley & Sons; 1997: 174-186.
6. Manisha Panda, Vasudha Dadi, Srinivasa Rao, Yarraguntla Vara Prasad Rao K. RP-HPLC method for determination of Azelnidipine and Telmisartan in pharmaceutical dosage form, Research Journal of Pharmacy and Technology 2023; 16(2); 509-3, doi: 10.52711/0974-360X.2023.00086.
7. M. S. Mohammed, "Spectrophotometric method for the estimation of clinidipine in bulk and pharmaceutical dosage forms," *Oriental Journal of Chemistry*, vol. 29, no. 1, pp. 131–134, 2013.
8. D. Basava Chaitanya, & M. Ajitha. (2022). Stability Indicating RP-HPLC Method Development and Validation for simultaneous estimation of Azelnidipine and Telmisartan in Bulk and Pharmaceutical Dosage Form: <https://doi.org/10.54037/WJPS.2022.100113>. World Journal of Pharmaceutical Sciences, 10(01), 121–127.
9. Akanksha, Shirsath; Manish, Raskar; Ganesh, Barkade; Varsha, Raut; Dnyaneshwar, Sangale. Simultaneous Estimation of Metoprolol Succinate, Telmisartan and Cilnidipine In Bulk Pharmaceutical Dosage Form. International Journal of Chemical & Pharmaceutical Analysis, 2021, Vol 8, Issue 4, p1.
10. P. Ravi Sankar, V. Swathi and P. 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. IJPSR (2019), Volume 10, Issue 4.
11. Dr. Bakhshi Abdurrahman, Ahire Kiran, Dr. Umair Ahmed, Ambreen Kauser, Dr. Deshmukh Tarique, Dr. Rashid Akhtar. Development And Validation of Stability Indicating Rp-Hplc Method for Estimation of Cilnidipine and Olmesartan in Bulk and Pharmaceutical Dosage Form. Afr.J.Bio.Sc. 6(13) (2024) 1169-1180.
12. Syed Hafeez Ahmed, Asra Jabeen, Riyaz Fatima, Fasi Ahmed Mohd, R. Sridhar Babu. A Validated Rp-Hplc Method for Simultaneous Estimation of Cilnidipine and Valsartan in Pharmaceutical Dosage Form. International Journal of Advanced Research in Medical & Pharmaceutical Sciences (IJARMPS-ISSN-2455-6998) Volume.6, Issue.3, June.2021.
13. Pravin Y. Khandagale, Nitin S. Bhajipale, Amol V. Badkhal. Rp-Hplc Method Development and Validation for Simultaneous Estimation of Cilnidipine and Telmisartan in Combined Pharmaceutical Dosage Form. International Research Journal of Pharmacy, 2017, 8 (9).
14. The Indian Pharmacopoeia, Government of India, Ministry of Health and Family welfare; 7th ed., The Indian Pharmacopoeia Commission, Ghaziabad, II: 2001; 1304-1305
15. Japanese Pharmacopoeia, 17th Edition, The Ministry of Health Labour and Welfare, 2016; 704-707.