International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Method Development and Validation of Simultaneous Estimation of Olmesartan Medoximil and Cilnidipine by Rp-HPLC in Pharmaceutical Dosage Form

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Abstract

In order to develop a Liquid Chromatographic method effectively, most of the effort should be spent in method development and optimization as this will improve the final method performance. The method validations documents for its intended purpose. A fast isocratic HPLC-PDA method was developed for the estimation of Olmesartan Medoximil and Olmesartan in tablet dosage form. The total run time was 8 minutes. Simple buffer was used. The mobile phases were easy to prepare and economical. The baseline with low signal to noise ratio was obtained. Each peak eluted with good resolution and no carryover was observed. Since the solutions were stable only for 24 hrs at room temperature. The purity of each peak was observed in PDA, from which an inference can be drawn that there is no interference of other peaks in the particular peak. The method allows high samples through put due to short run time. Thus, selective, simple and rapid method was developed. The method was validated with different analytical performance characteristics and data was compiled. The results satisfied the acceptance criteria. This demonstrated that the HPLC method developed was specific, linear, accurate, precise and robust. This work provided me a great opportunity to have an exposure with the instruments used in Pharmaceutical Industry.

Keywords: HPLC-PDA, Olmesartan Medoximil, Cilnidipine Chromatography

Full length article **Corresponding author's* e-mail: pharmarxpro@gmail.com

1. Introduction

Olmesartan is used to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. Olmesartan belongs to a class of drugs called angiotensin receptor blockers (ARBs). It works by relaxing blood vessels so that blood can flow more easily. Olmesartan belongs to the angiotensin II receptor blocker (ARB) family of drugs, which also includes telmisartan, candesartan, losartan and irbesartan. ARBs selectively bind to angiotensin receptor 1 (AT1) and prevent the protein angiotensin II from binding and exerting its hypertensive effects, which include vasoconstriction, stimulation and synthesis of aldosterone and ADH, cardiac stimulation, and renal reabsorption of sodium, among others. Cilnidipine belongs to a class of medicines called calcium channel blockers which are primarily taken for the treatment of hypertension (high blood pressure) and angina (chest pain). Cilnidipine is a diesterified 1,4-dihydropyridine-3,5-dicarboxylic acid. A calcium channel blocker, it is used as an antihypertensive. It has a role as a calcium channel blocker, an antihypertensive agent and a cardiovascular drug. It is a dihydropyridine, a 2-methoxyethyl ester and a C-nitro compound.

2. Materials and Methods

2.1. Equipment

Chromatographic separation was conceded on WATERS HPLC system which is outfitted with the 515 dual head reciprocating pump & a 2489 UV Visible detector. The software used is Empower-2 software and Phenomenex kinetex C18 (250mm×4.6mm i.d, 5µm) column is used for the investigation.

2.2. Chemicals and reagents

Olmesartan and Cilnidipine drugs were gifted by Aurobindo Pharmaceuticals, Hyderabad, Telangana, India.

Acetonitrile, methanol, HPLC grade water and mono sodium hydrogen orthophosphate and di sodium hydrogen ortho phosphate were procured from local manufacturers.

2.3. Selection of Detection wavelength

10 mg of Olmesartan and Cilnidipine was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay y spectrum was used for selection of wavelength for Olmesartan and Cilnidipine. The isosbestic point was taken as detection wavelength.

2.4. Selection of column

Column is selected based on solubility, polarity and chemical differences among Analytes [Column: Agilent C18 (4.6 x 250mm, 5µm].

2.5. Selection of mobile phase

Methanol: ACN (70:30%v/v) has been selected as mobile phase. If any buffer selected buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved.

2.6. Preparations and procedures

2.6.1. Preparation of mobile phase:

A mixture of Methanol 700ml (70%), 300 mL of ACN (30%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

2.6.2. Diluent Preparation

Mobile phase is used as Diluent.

2.6.3. Olmesartan standard preparation

10mg of Olmesartan working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluent.

2.6.4. Preparation of the individual Cilnidipine standard

10mg of Cilnidipine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluent.

2.6.5. Preparation of Sample Solution (Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Olmesartan and Cilnidipine (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of diluents is added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted up to the mark with diluent.

- ***Procedure***

20µL of the standard, sample are injected into the chromatographic system and the areas for Olmesartan and Cilnidipine peaks are measured and the %Assay are calculated by using the formulae.

2.8. System Suitability

Tailing factor for the peaks due to Olmesartan and Cilnidipine in Standard solution should not be more than 2.0.

Theoretical plates for the Olmesartan and Cilnidipine peaks in Standard solution should not be less than 2000

2.9. Preparation of standard stock solution

Accurately 10 mg of Olmesartan and 10mg of Cilnidipine working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further this Stock was pipette (3ml and 0.3ml) into a 10ml volumetric flask and dilute up to the mark with diluents.

- ***Procedure***

The standard solution was injected for five times and the area for all five injections measured in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

3. Results and Discussion

3.1. Selection of detection wavelength

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Olmesartan and Cilnidipine was obtained and the isobestic point of Olmesartan and Cilnidipine showed absorbance's maxima at 239 nm shown in figure 3. The chromatographic method development for the simultaneous estimation of Olmesartan and Cilnidipine were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of Olmesartan and Cilnidipine in API and pharmaceutical dosage form by RP-HPLC method.

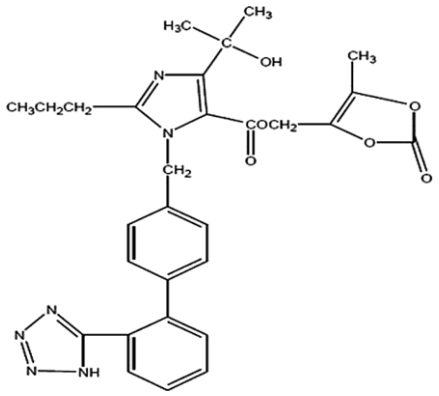

Figure 1: Chemical structure of Olmesartan

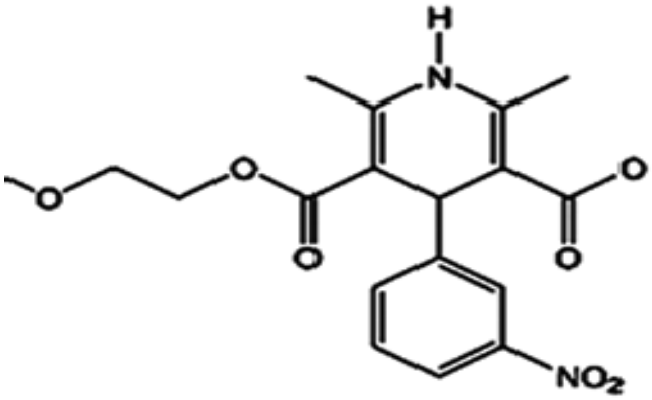

Figure 2: Chemical structure of Cilnidipine

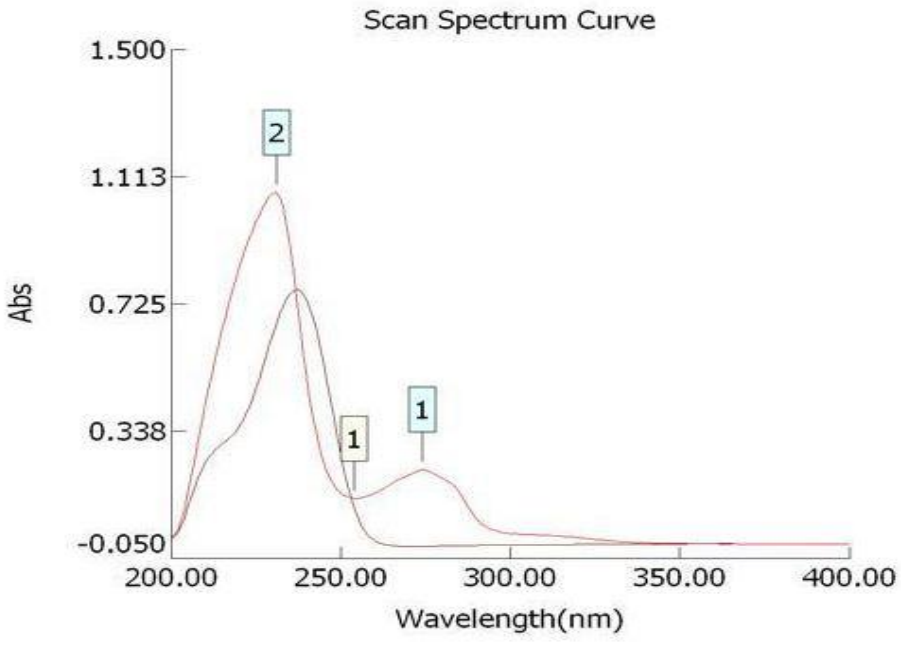

Figure 3: Standard graph of Cilnidipine

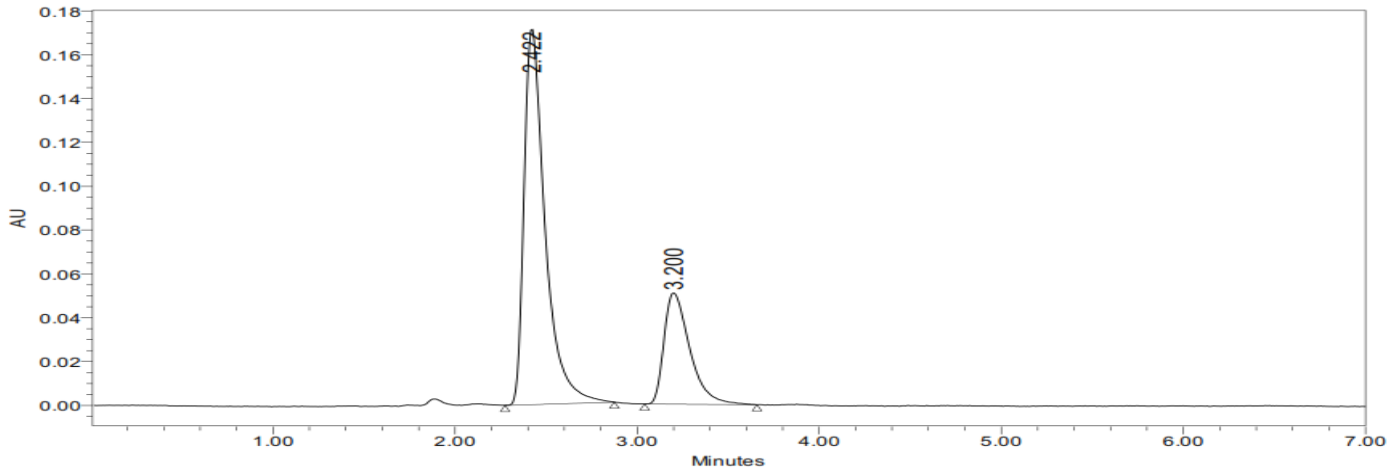

Figure 4. Chromatogram for Robustness more organic

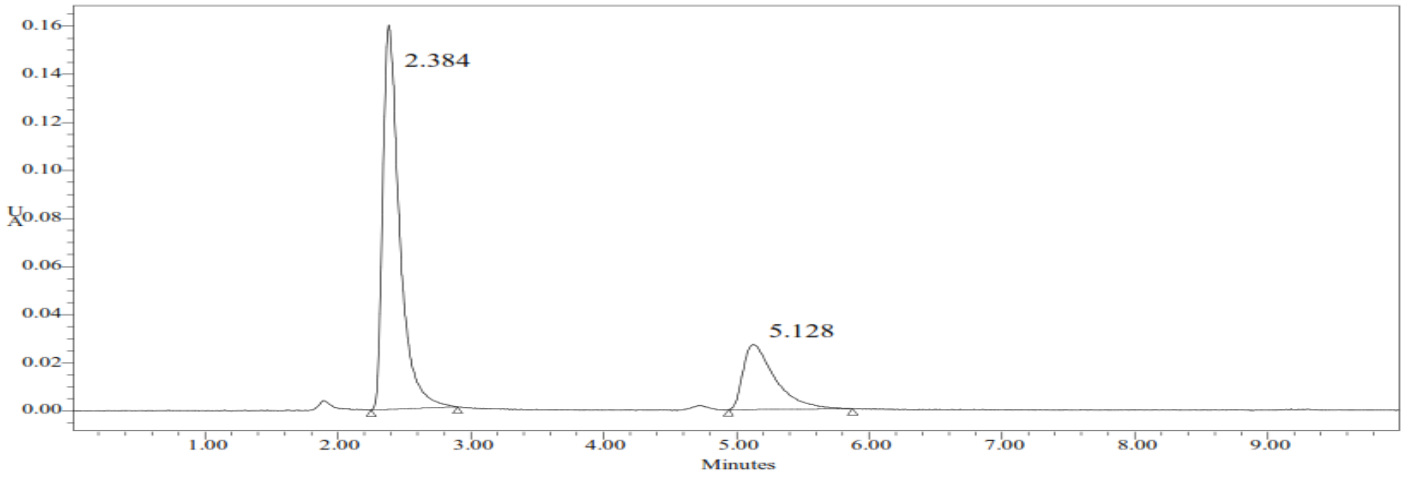

Figure 5. Chromatogram for Robustness less organic

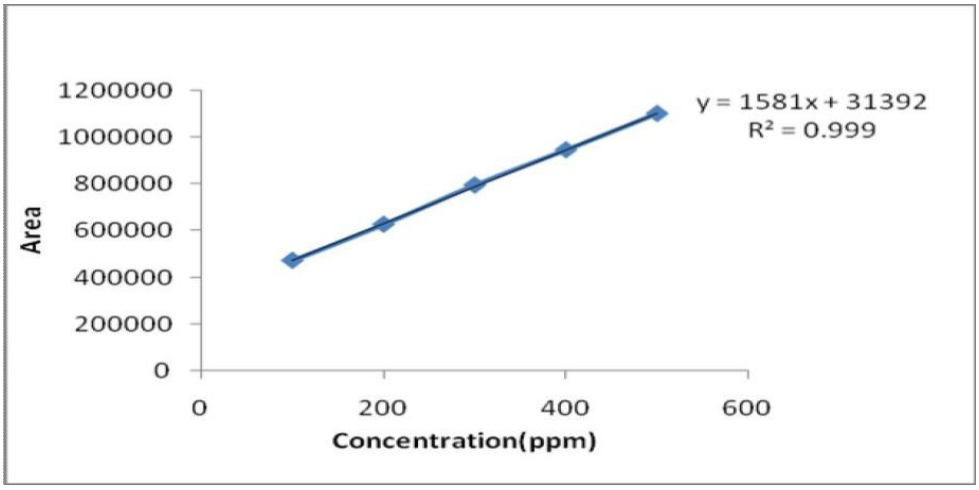

Figure 6. Calibration curve of Olmesartan

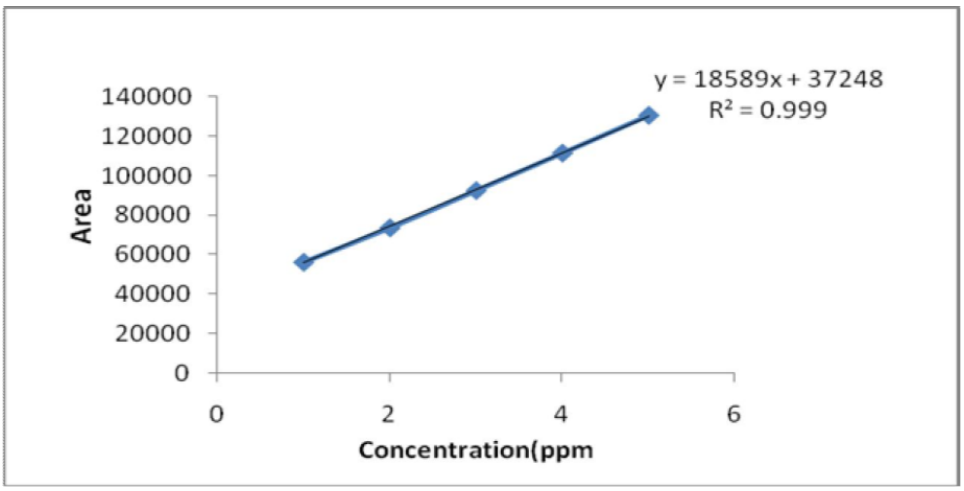

Figure 7. Calibration curve of Cilnidipine

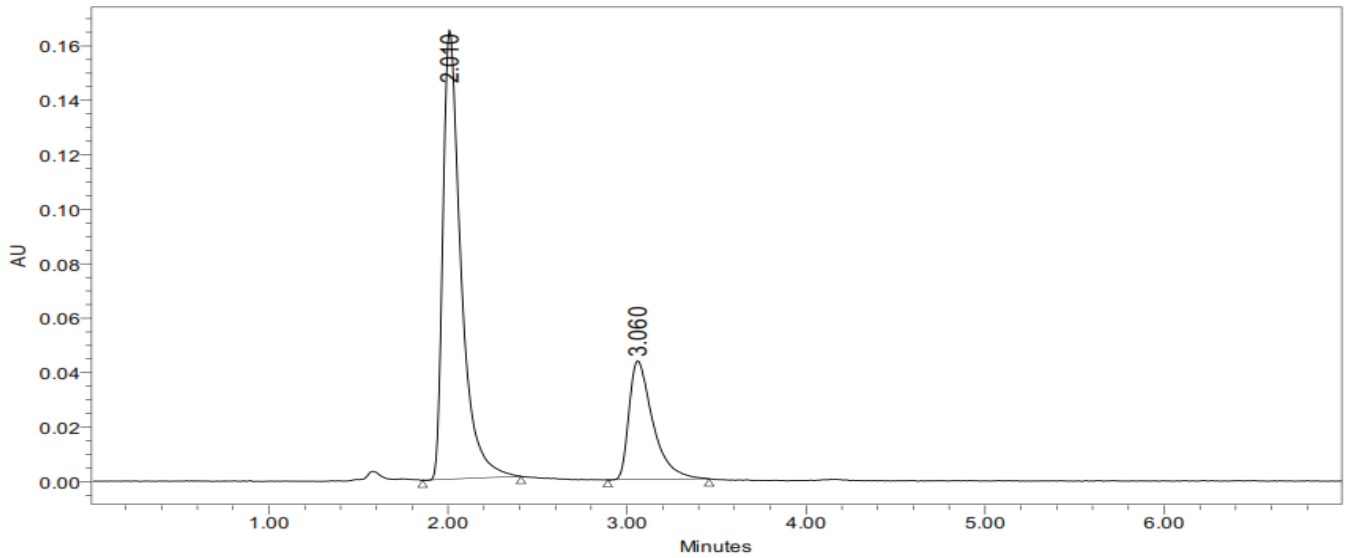

Figure 8. Chromatogram for Robustness more flow

Table 1. System suitability results For Olmesartan (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.9	3436	1.6
2	1.1	2831	1.7
3	1.3	2613	1.8

* Results for actual flow (1.0 ml/min) have been considered from Assay standard

Table 2. System suitability results for Cilnidipine (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	2158	1.8
2	1.0	2114	1.7
3	1.2	2069	1.7

* Results for actual flow (1.0ml/min) have been considered from Assay standard

Table 3. Details of Robustness more organic

	Name	RT	Area	Height (µv)	Usp plate count	Usp taililng
1	Olmesartan	2.422	1378798	171546	2358.0	1.7
2	Cilnidipine	3.200	499679	50843	2616.1	1.6

Table 4. Details of Robustness less organic

	Name	RT	Area	Height (µv)	Usp plate count	Usp taililng
1	Olmesartan	2.384	1404976	159808	2910.4	1.8
2	Cilnidipine	5.128	453297	27049	2840.1	1.7

Table 5. System suitability results for Olmesartan (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	2910	1.8
2	Actual	2860	1.7
3	10% More	2358	1.7

* Results for actual Mobile phase composition (55:45Buffer: Methanol) have been considered from Accuracy standard

Table 6. System suitability results for Cilnidipine (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	2540	1.7
2	Actual	2458	1.7
3	10% More	2616	1.7

* Results for actual Mobile phase composition (55:45Buffer: Methanol) have been considered from Accuracy

Table 7: Linearity Results

	Name	RT	Area	Height (µv)
1	Olmesartan	2.297	869216	109198
2	Olmesartan	2.264	1148093	145069
3	Olmesartan	2.308	1398858	164962
4	Olmesartan	2.370	1676584	193291
5	Olmesartan	2.322	1936686	238262
6	Cilnidipine	3.458	296156	30269
7	Cilnidipine	3.351	371946	39434
8	Cilnidipine	3.488	452984	45638
9	Cilnidipine	3.712	537383	50538
10	Cilnidipine	3.535	617463	65483

Table 8: Details of Robustness more flow

	Name	RT	Area	Height (µv)	Usp plate count	Usp taililng
1	Olmesartan	2.010	1150303	165118	2069.9	1.7
2	Cilnidipine	3.060	402322	43574	2713.8	1.7

Table 9. Details of Robutness less flow

	Name	RT	Area	Height (µv)	Usp plate count	Usp taililng
1	Olmesartan	2.960	1690740	161204	2158.1	1.8
2	Cilnidipine	5.244	519208	36602	3536.2	1.7

3.2. Optimized Chromatographic conditions

- Column: Phenomenex kinetex C18 (250mm×4.6mm i.d, 5µm) column.
- Mobile phase: Methanol: Mono and disodium Hydrogen orthophosphate buffer of pH 6.8: acetonitrile (47:23:30 %V/V)
- Flow rate: 1ml/min
- Injection volume: 20µl
- Detection wavelength: 287nm
- Mode of elution: Isocratic
- Column temperature: Ambient

3.3. Validation of the method [7-10]

System suitability test: Solution for system suitability test was all set by moving 1ml of standard stock arrangement (1000µg/ml) into 10ml volumetric flagon, weakening to check with diluent and sonicated. This preparation was injected six times into the HPLC system for assessing parameters like number of hypothetical plates (N), peak area and tailing factor. The results were shown in table 1 and the overlain chromatogram for system suitability was shown in figure 3.

3.4. Mobile Phase

The Organic composition in the mobile phase was varied from 70% to 60%. Standard solution 300 µg/ml of Olmesartan & 3µg/ml of Cilnidipine was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method. The results are summarized. On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10

3.5. Linearity

Working standard solution was prepared according to the procedure and after filtering and sonicating the solution for 5mins further dilutions were made to get different concentration levels ranging from 20 to 300µg/ml. Every solution was injected into HPLC system as well as linearity was appraised. The calibration curve was designed taking concentration on X-axis along with peak area on Y-axis. The linearity plots were shown in figure 6 and 7.

3.6. Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature

Variation was made to evaluate the impact on the method.

4. Conclusion

In order to determine Olmesartan and Cilnidipine simultaneously, a unique approach based on RP-HPLC was devised. To separate Olmesartan, an Agilent C18 column (4.6 x 150 mm, 5), and a detection wavelength of 254 nm were determined to be optimal. The calculated retention times were 2.34 and 3.28 hours. Olmesartan and Cilnidipine were both found to be 99.97% pure, whereas Cilnidipine was found to be 101.27 percent pure. In a linearity investigation spanning 50–250 mg of Cilnidipine and 5–50 mg of Olmesartan, respectively, we found recoveries of 99.56 and 99.48%, respectively. Intermediate precision had an RSD of 0.1, while repeatability was 0.2. Reliability and repeatability of the research were not compromised in any way. The LOQ values varied from 0.0172 to 0.2125, whereas the LOD values were 3.17 and 5.68.

Acknowledgements

The authors acknowledge Aurobindo Pharmaceuticals Limited, Hyderabad, Telangana, India for providing gift sample of Itraconazole and Terbinafine.

Conflict of interest

The authors declare that they have no conflict of interests.

References

[1] Chinese Pharmacopoeia Commission, Pharmacopoeia of the People's Republic of China, Vol. 1, China Medical Science Press, Beijing, 2010, p. 334.

[2] M.T. Rao et al, (2015). Development and validation of RP-HPLC method for the determination of itraconazole in bulk and capsule dosage form. International Journal of Pharmaceutical Sciences.

[3] D.T. Ha, T.N. Trung, T.T. Phuong, N.H. Yim, Q.C. Chen, and K. Bae. Bioorg. Med.Chem. Lett., 20, 6076–6081 (2010).

[4] R.R. Raju et al, (2011). Simultaneous Analysis Of RP-HPLC Method Development and Validation Of Terbinafine And Bezafibrate Drugs. Pharmaceutical Dosage Form. 2(4): 195-201.

[5] Q. Chang, G.N. Wang, Y. Li, L. Zhang, C. You, and Y. Zheng, Phytomedicine, 19,1024–1028 (2012).

[6] M. Zeng, Z. Lu, S. Yang, M. Zhang, J. Liao, S. Liu, X.J. Teng. (1996). Chromatography B. 681: 299–306.

[7] B. Ramu, K.K. Chandrul, P.S. Pandiyan. (2019). BioAnalytical Method Development of Repaglinide Drug Delivery Systems. Journal of Drug Delivery and Therapeutics. 9(6): 140-142.

[8] S.P. Singh, R.S.P. Singh, Wahajuddin, and G.K. Jain, J.Chromatogr.B, 876, 1–7(2008).

[9] E.M. Hodel, B. Zanolari, T. Mercier, J. Biollaz, J. Keiser, P. Olliaro, B. Genton. (2009). Journal of Chromatography B. 877: 867–886.

[10] I.C. César, J.A. De Aquino Ribeiro, L. De Souza Teixeira, K.B. Bellorio, F.C. De Abreu, J.M. Moreira et al, (2011).Journal of Pharmaceutical and Biomedical Analysis. 54: 114-120.

[11] Wahajuddin, S.P. Singh and G.K. Jain. J. Chromatogr. B, 877, 1133–1139 (2009).

[12] P.J. Taylor. Clini. Biochem., 38, 328–334 (2005).