Research Article

Ultra Violet Spectrophotometric Method Development and Validation for Simultaneous Quantification of Azelnidipine and Telmisartan in Pharmaceutical Dosage Form

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ABSTRACT

A simple, economical, accurate, precise and less time-consuming UV Spectrophotometric method has been developed and validated for simultaneous estimation of Azelnidipine and Telmisartan in bulk and pharmaceutical dosage form. In this method Azelnidipine and Telmisartan are exhibits maximum absorbance (λ max) at 245 nm and 296 nm with methanol as the solvent. The method was validated as per the International Conference on Harmonization (ICHQ2R1) guidelines. Drugs followed the linearity in the concentration range of 1-6 µg/mL and 5-30 µg/mL with correlation coefficient (r^2) of 0.9999 and 0.9987 for Azelnidipine and Telmisartan respectively. The validity of the proposed method was assessed by applying the standard addition technique where the percentage recovery of the added standard was found to be 100.17 and 99.86 for Azelnidipine and Telmisartan. The limit of detection and quantification were calculated and found to be 0.1147 µg/mL and 0.3475 µg/mL and 2.1825 µg/mL and 6.6137 µg/mL for Azelnidipine and Telmisartan respectively. The proposed method is recommended for routine analysis of Azelnidipine and Telmisartan in bulk and pharmaceutical dosage forms in regular quality control testing laboratories.

Keywords: Azelnidipine, Telmisartan, UV Spectrophotometry, Beer's Law, Validation.

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INTRODUCTION

Hypertension (HT) is a very common disorder, particularly for past middle age. It is not a disease in itself, but is an important risk factor for cardiovascular mortality and morbidity. Hence, a fixed dose combination of Azelnidipine and Telmisartan was approved by US-FDA for the treatment of hypertension. The fixed dose combination of Azelnidipine and Telmisartan is safe, well tolerated, with lower incidence of adverse effects compared to that observed with monotherapy of Azelnidipine.

Azelnidipine (AZEL) is a dihydropyridine type of Calcium channel blockers used for the treatment of hypertension and angina pectoris. Chemically it is 3-[1-(Benzyldrylazetidin-3-yl]5-isopropyl-2-amino 6 methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3, 5 dicarboxylate; It is freely soluble in acetone and acetic acid, soluble in methanol, ethanol and ethyl acetate and practically insoluble in water. It acts by inhibiting trans membrane Ca^{2+} influx through the voltage dependent channels of smooth muscles in vascular walls [1-4].

Telmisartan (TEL) belongs to Angiotensin receptor blockers with chemical name 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1yl] methyl] phenyl] benzoic acid. It is soluble in strong base & methanol, and sparingly soluble in strong acid (except HCL). It interferes with the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. Angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of an aldosterone blockage of its effects results in decrease in systemic vascular resistance [5-6].

The literature survey revealed that some UV [7-15] and RP-HPLC[16-38] methods were reported for the determination of AZEL and TEL individually and fixed dose combination of AZEL and TEL in bulk and pharmaceutical dosage form. Present study aimed to develop new UV Spectrophotometric method and validation for the determination of AZEL and TEL in bulk and in its pharmaceutical dosage form with good accuracy and precision. The developed method was validated according to ICH Q2R1 analytical method validation parameters [39-40]. Chemical structure of AZEL and TEL are shown in Figure 1.

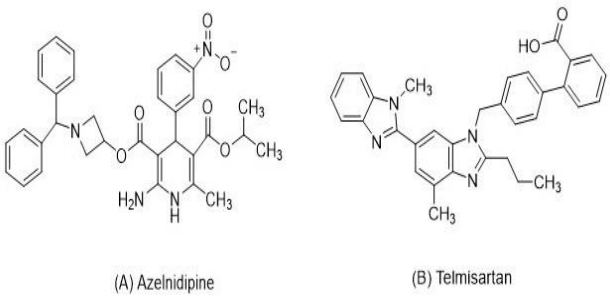

Figure 1: Chemical Structures of (A) Azelnidipine and (B) Telmisartan

MATERIALS AND METHODS

Chemicals and Reagents

Azelnidipine (≥98%), was bought from Aavyan Labs, Hyderabad, India. and Telmisartan (≥98%) standard drugs (API) were obtained as gift samples from Dr. Reddy's Laboratories, Hyderabad, India. HPLC grade Methanol was purchased from Thermo Fisher Scientific India Pvt. limited, Mumbai, India. Water purified by the Milli-Q water purification system was used in the study. The rest of the chemicals and reagents were procured from standard commercial supplier.

Equipment

Shimadzu (UV-1780) Double beam UV-Visible spectrophotometer with 1cm matched quartz cells was used for the measurement of absorbance. Shimadzu-AX-200 electronic balance was used for weighing the samples. Citizen-Ultrasonicator and Class 'A' volumetric glassware's were used.

Selection of Solvent for Analysis

In the present study the UV spectra of AZEL and TEL were obtained from different solutions (Methanol, Acetonitrile, Distilled water) were studied. The two drugs were freely soluble in Methanol. At the end of these studies, Methanol was chosen as solvent for studied drugs.

Selection of detection wavelength for simultaneous estimation

In the present study the drug solutions of AZEL (5 µg/mL) and TEL (25 µg/mL) were prepared and scanned over a range of 200-400 nm. It was observed that the drugs showed maximum absorbance at 245 and 271 nm for AZEL and 296 nm for TEL. The overlay spectra of AZEL and TEL (5 µg/mL and 25 µg/mL) is shown in Figure 2. which was chosen as the detection wavelength (Isosbestic wavelength 245 nm) for the determination of both analytes.

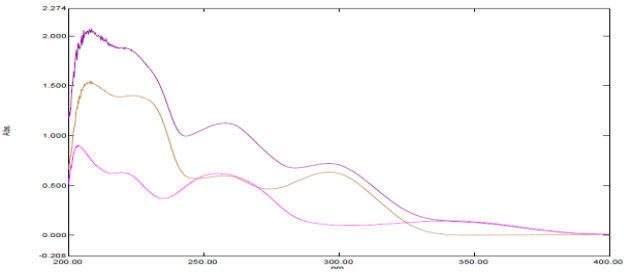

Figure 2: Overlay Spectra of Azelnidipine and Telmisartan (5 µg/mL & 25 µg/mL)

Preparation of standard stock solutions of analytes

Primary stock solutions of AZEL and TEL were prepared separately by dissolving accurately weighed 10 mg of each pure drug samples in methanol in 100 mL volumetric flasks and made the volume up to the mark using same solvent to produce final concentration of 100 µg/mL each of AZEL and TEL respectively.

Calibration curve

Appropriate aliquots 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mL of prepared working standard solutions of AZEL and 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL TEL were transferred into series of 10 mL volumetric flasks and diluted and made up to the mark with methanol to obtain final concentration of 1-6 µg/mL and 5-30 µg/ml of AZEL and TEL respectively. The above solutions were scanned over the range of 200 nm to 400 nm against reagent blank. The absorbances of each solution were measured at 245 nm against methanol as blank. A calibration curve was prepared by plotting absorbance versus concentration.

Estimation of Azelnidipine and Telmisartan in Tablets

For the analysis of dosage form, twenty tablets of Uniaz T 40 (Azelnidipine-8 mg and Telmisartan- 40 mg) were ground to fine powder and mixed thoroughly. A quantity of powder equivalent to one tablet weight of each of the drug was transferred to 100 mL volumetric flask and dissolved in about 20 mL methanol sonicated for 10 min and volume was made up to mark with the same solvent. The insoluble excipients were separated by filtration through Whatman filter paper. After suitable dilution, absorbance of the prepared sample solutions (5 µg/mL and 25 µg/mL) of Azelnidipine and Telmisartan was recorded against the reagent blank at 245 nm.

RESULTS AND DISCUSSION

Method validation was performed by following the International Conference on Harmonization (ICHQ2R1) guidelines.

Linearity

Fresh aliquots were prepared from standard stock solution ranging from 1-6 µg/mL and 5-30 µg/mL of AZEL and TEL. The absorbance values of each concentration were recorded at 245 nm. For this study methanol was used as a blank. Results of Linearity study were shown in Table 1 and Figure 3.

Table 1: Linearity results of Azelnidipine and Telmisartan

Azelnidipine		Telmisartan	
Concentration (µg/mL)	Absorbance	Concentration (µg/mL)	Absorbance
1	0.072	5	0.079
2	0.148	10	0.174
3	0.223	15	0.275
4	0.298	20	0.381
5	0.377	25	0.482
6	0.453	30	0.575

Accuracy

Accuracy of the developed method was confirmed by performing recovery studies at three different concentration ranges, each one in triplicate. From the recovery studies it was clear that the method remains very

accurate for quantitative estimation of tablet as the statistical results were within the acceptance range. Results of accuracy study were shown in Table 2.

Precision

The intraday and interday precision were executed by analyzing six independent analyses 3 µg/mL and 15 µg/mL for Azelnidipine and Telmisartan. The standard deviations, relative standard deviation was calculated and results of the study are acceptable and can be considered to be very reasonable the results are summarized in Table 3 and Table 4.

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification of Azelnidipine and Telmisartan by proposed method were determined using calibration curve. LOQ and LOD were calculated as

LOD = 3.3 X S.D/S

LOQ = 10 X S.D/S

Where S is the slope of the calibration curve and SD is the standard deviation of response of least concentration of calibration curve in three replicates. The limit of detection and quantification were calculated and found to be 0.1147 µg/mL and 0.3475 µg/mL and 2.1825 µg/mL and 6.6137 µg/mL for Azelnidipine and Telmisartan respectively. The results shown that sensitivity of the proposed method.

Robustness

Robustness of the method was determined by carrying out the analysis at three different wavelengths (±2 nm). The respective absorbance was noted and the result was indicated by % RSD. Results of the study were shown in Table 5.

Application of the proposed method to tablet dosage form

The proposed methods were applied to the quantification of Azelnidipine and Telmisartan in tablet dosage forms. The results shown in Table 6, suggest that the method is suitable for the determination of Azelnidipine and Telmisartan with good accuracy and precision. The excipients in the dosage forms do not interfere in the assay procedure.

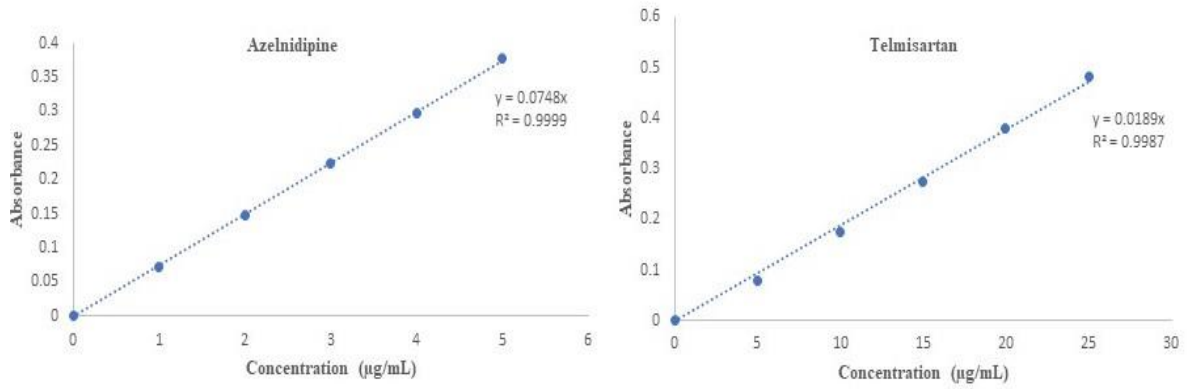

Figure 3: Linearity curves of Azelnidipine and Telmisartan

Table 2: Accuracy data of Azelnidipine and Telmisartan

S.No.	Azelnidipine				Telmisartan			
	µg/mL added	µg/mL found	% Recovery	mean% recovery	µg/mL added	µg/Ml found	% Recovery	Mean% recovery
1	2	2	100	99.75	10	9.98	99.5	99.5
2	2	1.97	99.25		10	10	100	
3	2	2	100		10	9.96	99.0	
4	4	4.02	100.25	100.37	20	20	100	100.04
5	4	4	100		20	20.01	100.12	
6	4	4.07	100.87		20	20	100	
7	6	6.02	100.16	100.41	30	30	100	100.05
8	6	6.04	100.33		30	30	100.16	
9	6	6.09	100.75		30	30	100	

Table 3: Intra-day precision data of proposed method

S.No.	Azelnidipine		Telmisartan	
	Conc. (µg/mL)	Absorbance	Conc. (µg/mL)	Absorbance
1	3	0.223	15	0.275
2	3	0.222	15	0.274
3	3	0.221	15	0.273
4	3	0.223	15	0.275
5	3	0.221	15	0.274
6	3	0.222	15	0.275
Mean		0.222		0.274
SD		0.001		0.0008
%RSD		0.450		0.326

Table 4: Inter-day precision (Reproducibility) data of proposed method

S.No.	Conc. (µg/mL)	Azelnidipine			Conc. (µg/mL)	Telmisartan		
		Day 1	Day 2	Day 3		Day 1	Day 2	Day 3
1	3	0.220	0.216	0.224	15	0.269	0.273	0.267
2	3	0.219	0.217	0.222	15	0.267	0.272	0.265
3	3	0.218	0.215	0.223	15	0.268	0.271	0.266
4	3	0.220	0.217	0.223	15	0.268	0.273	0.265
5	3	0.218	0.216	0.224	15	0.269	0.272	0.267
6	3	0.219	0.215	0.222	15	0.267	0.271	0.266
Mean		0.219	0.216	0.223		0.268	0.272	0.266
SD		0.0008	0.0008	0.0008		0.0008	0.0008	0.0008
%RSD		0.408	0.414	0.401		0.333	0.328	0.336

Table 5: Robustness studies data of the Proposed Method

S. No.	Conc. (µg/mL)	Azelnidipine			Conc. (µg/mL)	Telmisartan		
		243 nm	245 nm	247 nm		243 nm	245 nm	247 nm
1	3	0.295	0.298	0.301	15	0.273	0.275	0.277
2	3	0.294	0.297	0.300	15	0.272	0.274	0.275
3	3	0.293	0.296	0.299	15	0.271	0.273	0.276
4	3	0.295	0.297	0.300	15	0.271	0.275	0.277
5	3	0.294	0.298	0.301	15	0.273	0.273	0.275
6	3	0.293	0.296	0.299	15	0.272	0.274	0.276
Mean	3	0.294	0.297	0.300		0.272	0.274	0.276
SD		0.0008	0.0008	0.0008		0.0008	0.0008	0.0008
%RSD		0.304	0.301	0.298		0.328	0.326	0.324

Table 6: Assay results of Azelnidipine and Telmisartan in tablet dosage form

Formulation	Label Claim (mg/tablet)	Amount Found (mg/tablet)	% Assay
Uniaz T 40	Azelnidipine-8	7.89	98.62
	Telmisartan- 40	39.73	99.32

CONCLUSION

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries of the drugs were obtained at each added concentration, which indicates that the method was accurate. The LOD and LOQ were found to be in microgram level, which indicates the sensitivity of the method. The method was also found to be robust as indicated by the %RSD values which are less than 2%. The results of assay shows that the amount of drugs was in good agreement with the label claim of the formulation as indicated by % assay. The proposed method also can be used for the routine quality control analysis of Azelnidipine and Telmisartan in bulk and pharmaceutical formulations.

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REFERENCES

1. The Indian Pharmacopoeia, Government of India, Ministry of Health and Family welfare; 7th ed., The Indian pharmacopeia commission, Ghaziabad, 2018; II: 1304-1305,3319-3320.
2. Goodman and Gilman's The pharmacological basis of therapeutics; 10th Edn; Medical publishing division, 2001; 1804.
3. Tripath KD. Essentials of Medical Pharmacology; 6th ed., New Delhi, Jaypee Brothers Medical Publishers Ltd., 271-275.
4. Drug Profile "Azelnidipine", December,2020. http://www.drugbank.ca/drugs/DB09230.
5. Japanese Pharmacopoeia, 17th Edition, The Ministry of Health Labour and Welfare, 2016; 704-707.
6. Drug Profile, "Telmisartan", December, 2020. http://www.drugbank.ca/drugs/DB00966.
7. Raskapur KD, Patel MM, Captain AD. UV-Spectrophotometric method development and validation for determination of Azelnidipine in pharmaceutical dosage form. Toxicology. 2010; 106:135-43.
8. Rele RV. Spectrophotometric estimation of Azelnidipine in bulk and pharmaceutical dosage form by second order derivative method. Journal of Chemical and Pharmaceutical Research. 2014;6(8):198-202.
9. Chivate ND, Patil SM, Saboji JK, Chivate AN. Development of UV spectrophotometric method for estimation and validation of telmisartan as a pure API. Journal of Pharmacy Research. 2012 Jun;5(6):3331-3.
10. Rathod SD, Patil PM, Waghmare SS, Chaudhari PD. UV-spectrophotometric method for estimation of telmisartan in bulk and tablet dosage form. International Journal of Pharmaceutical Sciences and Research. 2012 Oct 1;3(10):3936.
11. Kumar M, Kumar C, Bhatt S, Pandurangan A, Kaushik V, Malik A, Saini V. Dissolution method development and validation for tablet dosage form of Telmisartan using UV spectrophotometric method. J. Chem. Pharm. Res. 2018;10(5):148-56.
12. Jadhav RS, Ubale M. UV Spectrophotometric analytical method development and validation for determination of Telmisartan in Pharmaceutical Drug and Drug Product (Tablet dosage form): Int. J. of Current Advanced Research. 2018;7(6):13292-6.
13. S. Yuvasri, S. Murugan and T. Vetrichelvan, First- Order Derivative and UV-Spectrophotometric Methods for Simultaneous Determination of Telmisartan and Azelnidipine in Bulk and Tablet Dosage Form, European Journal of Biomedical and Pharmaceutical Sciences 2021; 8(5): 290-294.
14. Kumara Prasad S. A., Unnathi Y. Shetty, Vidya Shree and Pradeep, Development and Validation of UV Spectrophotometric Method for the Simultaneous Estimation of Azelnidipine and Telmisartan in Combined Dosage Form, World Journal of Pharmacy and Pharmaceutical Sciences 2021; 10(12): 1320-1332.
15. Prabhakar D, Sreekanth J, Jayaveera KN. Method Development and Validation of Azelnidipine by RP-HPLC: Int. J. of Chem. Tech. Research. 2017;10(10):418-23.
16. Gore MG, Dabhade PS. RP-HPLC method development and validation of Azelnidipine. International Journal of Pharmaceutical Sciences and Research. 2016 Dec 1;7(12):5111-5114.
17. Modi J, Patel SK, Parikh N, Shah SR, Pradhan PK, Upadhyay UM. Stability indicating analytical method development and validation for estimation of Azelnidipine. World J Pharm Res. 2016; 5:831-47.
18. Surekha ML, Swamy GK, Ashwini GL. Development and Validation of RP-HPLC method for the estimation of Telmisartan in bulk and tablet dosage Form. International Journal of Drug Development and Research. 2012;4(4):200-205.
19. Nandipati S., Reddy V. Development and Validation of RP-HPLC Method for Estimation of Telmisartan in Bulk and Tablet Dosage Form: Int. Research J. of Pharma. and App. Sci. 2012; 2(3): 39-43.
20. Patra BR, Mohan S, Gowda N. Stability-indicating RP-UHPLC method for determination of telmisartan in drug substance and marketed formulation. International Journal of Pharmaceutical Sciences and Research. 2016 May 1;7(5):2031-2039.
21. Sujana K, Gowri Sankar D, Bala Souri O, Swathi Rani G. Stability indicating RP-HPLC method for the determination of telmisartan in pure and pharmaceutical formulation. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3(2):164-167.
22. Bhadoriya U., Dhaked H., Upendra et al. RP-HPLC method Development and validation for estimation of Telmisartan in bulk and tablet dosage form: Int. J. of drug Reg. Affairs. 2013; 1(12): 61-64.
23. A. Gupta, R. M. Charde and M. S. Charde. Determination of Telmisartan and forced degradation behaviour by RP-HPLC in tablet dosage form: Journal of Pharmacy Research .2011;4(4):1270-1273.
24. Ch. Phani Kishore, V. Bhanu Prakash Reddy, Dhanashri M Kale. Development and validation of stability indicating HPLC

method for the estimation of Telmisartan related substances in tablets formulation: International Journal of Research and Pharmaceutical Sciences.2010;1(4): 493-501.

25. D. Basava Chaitanya, M. Ajitha. Stability Indicating RP-HPLC Method Development and Validation for simultaneous estimation of Azelnidipine and Telmisartan in Bulk and Pharmaceutical Dosage Form: World Journal of Pharmaceutical Sciences.2022;10(01): 121-127.
26. Kumar M, Chandra U, Garg A, Gupta P. Impurity profiling of Azelnidipine and Telmisartan in Fixed Dose Combination using Gradient RP-HPLC Method. Annals of the Romanian Society for Cell Biology. 2021 May 6:15050-67.
27. Snehal D. Jadhav, Prachi B. Lokhande, et al. Method Development & Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation for Azelnidipine & Telmisartan in Bulk & Pharmaceutical Dosage Form: World Journal of Pharmaceutical and Medical Research.2022; 8(3): 216 – 222.
28. Manish Kumar, Umesh Chandra, Arun Garg, Pankaj Gupta. Impurity profiling of Azelnidipine and Telmisartan in Fixed Dose Combination using Gradient RP-HPLC Method: Annals of the Romanian Society for Cell Biology 2021; 25(4): 15050-15067.
29. Agrawal S, Nizami T. Method Development and Validation for the Simultaneous Determination of Azelnidipine and Telmisartan in Tablet Dosage form by RP – HPLC: Int. Journal of Pharmaceutical Sciences and Medicine (IJPSM) 2021; 6(10): 26-36.
30. K.V.L.D. Spandana, N.J.P. Subhashini, Telmisartan and Azelnidipine Quantification Employing HPLC Stratagem; Stability Investigation on Telmisartan and Azelnidipine, International Journal of Applied Pharmaceutics 2022; 14(1): 261-265.
31. Krishna phanisri Ponnekanti, Sunitha, Development of HPLC Stability Demonstrating Methodology for Quantifying Azelnidipine and Telmisartan in Tablets and Bulk Types: Validation Following ICH Directives, International Journal of Applied Pharmaceutics 2021; 13(5): 298-305.
32. Kumar M, Chandra U, Garg A, Gupta P. Impurity profiling of Azelnidipine and Telmisartan in Fixed Dose Combination using Gradient RP-HPLC Method. Annals of the Romanian Society for Cell Biology. 2021 May 6:15050-67.
33. Basava Chaitanya D, Ajitha M, Stability Indicating RP-HPLC Method Development and Validation for simultaneous estimation of Azelnidipine and Telmisartan in Bulk and Pharmaceutical Dosage Form, World Journal of Pharmaceutical Sciences 2022; 10(01): 121-127.
34. Spandana V, Siddartha , Analytical Method Development and Validation of Azelnidipine and Telmisartan by RP HPLC Method, Journal of Pharmaceutics and Nanotechnology 2022; 10(2): 1-11.
35. Kumar M, Chandra U, Garg A, Gupta P. Development and Validation of In-vitro dissolution test using RP-HPLC Analysis for simultaneous estimation of Azelnidipine and Telmisartan in a Fixed-dose Combination. Research Journal of Pharmacy and Technology. 2022 May 30;15(5):1967-72.
36. Parikh Mansi Brijeshbhai, Dr. Pankti Dalwadi, Ms. Neetu Dharu, Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Telmisartan and Azelnidipine in Tablet Dosage Form, International Journal of All Research Education and Scientific Methods 2021; 9(5): 1082-1090.
37. Dange Shital Shrirang, Kalyankar Tukaram Mohanrao, Method Development and Validation for the Simultaneous Estimation of Telmisartan and Azelnidipine in Bulk and Tablet Dosage Form by Using HPLC, Science, Technology and Development 2020; 9(6).
38. Q2A: Text On; (1995) Validation of Analytical Procedures. In International Conference on Harmonization. Federal Register, 60(40):11260-11262.
39. Q2B: Text On; (1997) Validation of Analytical Procedures. In International Conference on Harmonization. Federal Register, 62(96):27463-27467.
40. P. Roja, M.M. Eswarudu, P. Ravi Sankar, P. Srinivasa Babu. An Updated Review on Analytical Methods for Estimation of Azelnidipine and Telmisartan. Asian Journal of Pharmaceutical Research and Development. 2022; 10(2): 59-76.

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