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Development and Validation of an RP-HPLC Method for Simultaneous Quantification of Azelnidipine and Metoprolol Succinate in Synthetic Mixtures

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ABSTRACT

This study outlines an RP-HPLC technique for quantifying azelnidipine and metoprolol succinate in a synthetic mixture. Azelnidipine, a calcium channel blocker, and metoprolol succinate, a beta-blocker, are both used for the management of hypertension. The method was optimized using a Shimadzu HPLC LC2010 system, equipped with a UV-VIS detector and a binary gradient system. A Hibar ODS C18 5 µm column (250 x 4.6 mm) was used for separation in isocratic mode, with a mobile phase consisting of methanol and water (70:30 v/v, pH 3.0), at a flow rate of 1.0 ml/min and detection set at 230 nm. The method demonstrated linearity for azelnidipine in the range of 8-40 µg/ml and for metoprolol succinate between 25-125 µg/ml. This developed method successfully determined both drugs in synthetic mixtures and followed the ICH Q2 R1 guidelines for method validation.

Keywords: Azelnidipine, Metoprolol succinate, RP-HPLC, Method validation, ICH guidelines

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Introduction

Azelnidipine and metoprolol succinate are both widely prescribed medications used to manage high blood pressure. Azelnidipine (AZL) is a calcium channel blocker that works by preventing the entry of calcium ions into the smooth muscle of blood vessels, which helps to relax and widen the vessels, leading to lower blood pressure and a slower heart rate [1]. On the other hand, metoprolol succinate is a beta-blocker that targets beta-1 receptors in the heart, reducing the heart rate and cardiac output, which aids in controlling hypertension and reducing the strain on the heart [2]. These two drugs are often used together in the treatment of stage 2 hypertension, a combination that was approved by the CDSCO on August 7, 2020. The recommended therapeutic doses are 16 mg of azelnidipine and 50 mg of metoprolol succinate. Although both drugs can be measured individually or in combination with other substances, no existing methods have been specifically developed to measure them together in a synthetic mixture [3-17]. Among the various analytical methods available, high-performance liquid chromatography (HPLC) is preferred due to its precision, speed, and effectiveness, making it the chosen technique for this study.

This study outlines an RP-HPLC technique for quantifying azelnidipine and metoprolol succinate in a synthetic mixture.

Materials and Methods

For this study, metoprolol succinate (99.78% pure) was kindly provided by Dwarkesh Pharmaceuticals Pvt Ltd., Vatva, while Azelnidipine (99.80% pure) was obtained from Pure Chem Pvt Ltd., Ankleshwar. The reagents used include Methanol, HPLC-grade water, and Orthophosphoric acid, sourced from Rankem Chemicals, Astron Chemicals, and Merck Chemicals, respectively.

Selection of detection wavelength

To select the optimal detection wavelength, solutions of metoprolol succinate (10 µg/mL) and azelnidipine (10 µg/mL) were prepared using methyl alcohol. The samples were then scanned across the UV range of 200-400 nm to identify the best wavelength for the analysis.

Instrumentation and chromatographic conditions

Working solutions of azelnidipine (10 µg/mL) and metoprolol succinate (10 µg/mL) were prepared in methyl alcohol. The UV scans were performed within the 200-400 nm range to help identify the ideal wavelength for detecting both compounds.

Preparation of standard stock solutions of AZL and MPL

For azelnidipine, 10 mg of the substance was accurately weighed and dissolved in methanol, making up a final volume of 100 mL to create a stock solution with a concentration of 100 µg/mL. A 1 mL aliquot of this solution was then diluted with the mobile phase to achieve a concentration of 10 µg/mL. Similarly, metoprolol succinate was prepared by weighing 10 mg and dissolving it in methanol, then diluting it to 100 mL to give a concentration of 100 µg/mL. This was further diluted with the mobile phase to give a final concentration of 10 µg/mL.

Preparation of standard stock solution of the mixture

A mixture of azelnidipine and metoprolol succinate was prepared by dissolving 16 mg of Azelnidipine and 50 mg of metoprolol succinate in methanol in a 100 mL volumetric flask. The concentrations in this stock solution were 160 µg/mL for azelnidipine and 500 µg/mL for metoprolol succinate. A 1 mL aliquot from this solution was then diluted with the mobile phase to achieve final concentrations of 50 µg/mL for metoprolol succinate and 16 µg/mL for azelnidipine.

System suitability parameters

The system suitability for the method was determined by injecting a sample solution containing both azelnidipine and metoprolol succinate (16 + 50 µg/mL). Key system parameters, such as retention time (Rt), tailing factor (T), resolution (Rs), and theoretical plate count, were evaluated by performing five injections of the sample. The relative standard deviation (RSD) was calculated to assess the consistency and reliability of the system. The method was validated following ICH guidelines [18], focusing on linearity, accuracy, precision, and limits of detection and quantification.

Linearity and range

To evaluate the linearity of the method, a stock solution was initially prepared by dissolving 16 mg of azelnidipine (AZL) and 50 mg of metoprolol succinate (MPL) in a 10 mL volumetric flask, achieving concentrations of 1600 µg/mL and 5000 µg/mL, respectively. Serial dilutions were then performed to obtain final concentrations ranging from 8–40 µg/mL for AZL and 25–125 µg/mL for MPL. Each solution was injected into the chromatographic system under optimal conditions, with an injection volume of 20 µL.

Repeatability

To determine the consistency of the method, standard solutions of AZL (8–40 µg/mL) and MPL (25–125 µg/mL) were injected five times at 20 µL each. Peak areas were recorded for each injection, and the relative standard deviation (RSD) was calculated to assess reproducibility.

Limit of detection (LOD) and limit of quantification (LOQ)

The lowest concentration at which AZL and MPL could be detected (LOD) and quantified (LOQ) was determined using two methods: (1) visual assessment and (2) a statistical approach based on response repeatability. The LOD and LOQ values were obtained using the mean slope and the standard deviation of the response.

Accuracy

The accuracy of the method was confirmed by spiking a placebo with known amounts of the drug mixture. Samples were prepared at three concentration levels—50%, 100%, and 150% of the target values (16 µg/mL for AZL and 50 µg/mL for MPL). Each level was analyzed three times, and the mean recovery percentage was calculated to validate accuracy.

Intraday and interday precision

To assess precision, analyses were performed at different time intervals within a single day (intraday) and across multiple days (interday). Three concentration levels were used (AZL + MPL = 8 + 25, 24 + 75, 40 + 125 µg/mL), ensuring that variations in analytical conditions did not significantly affect the results.

Robustness

Robustness was evaluated by introducing minor changes to the experimental conditions, including:

- Adjusting the flow rate by ± 0.05 mL/min (standard = 1.0 mL/min)
- Slightly modifying the pH of the mobile phase by ± 0.5 units (standard = pH 3.0)
- Altering the mobile phase composition by ± 5 mL

Each parameter variation was analyzed three times, and the RSD was calculated to assess method reliability.

Assay

For assay determination, a synthetic mixture containing 50 mg of MPL and 16 mg of AZL was prepared. The mixture was diluted, combined with a placebo, and adjusted to final working concentrations of 16 µg/mL for AZL and 50 µg/mL for MPL. Three separate injections of 20 µL were performed, and the percentage assay was calculated.

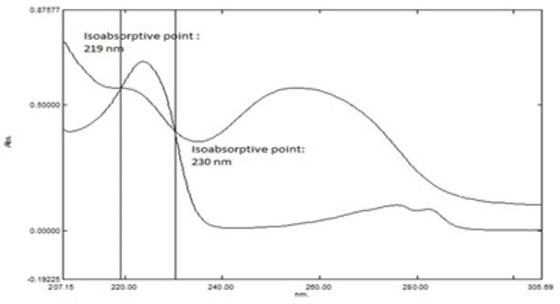
Chromatographic conditions

The analysis was carried out using a Shimadzu HPLC LC2010 equipped with a UV-VIS detector and a binary gradient system. Separation was performed on a Hibar ODS C18 (5 µm, 250 × 4.6 mm) column in isocratic mode, with methanol and water (70:30 v/v, pH 3.0) as the mobile phase, a flow rate of 1.0 mL/min, and UV detection at 230 nm.

Results and Discussion

Selection of analytical wavelength

When the absorption spectra of AZL and MPL were superimposed, two iso-absorptive points were identified at 219 nm and 230 nm. Among these, 230 nm was selected as the detection wavelength for quantifying AZL and MPL, as it provided optimal absorbance and sensitivity for analysis **(Figure 1)**.



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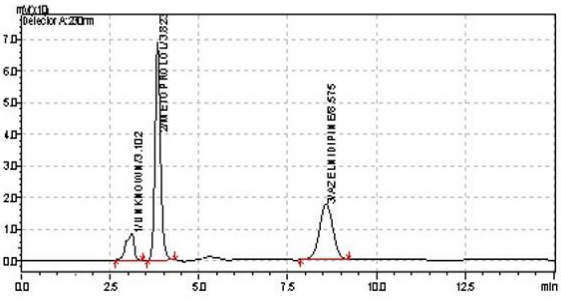

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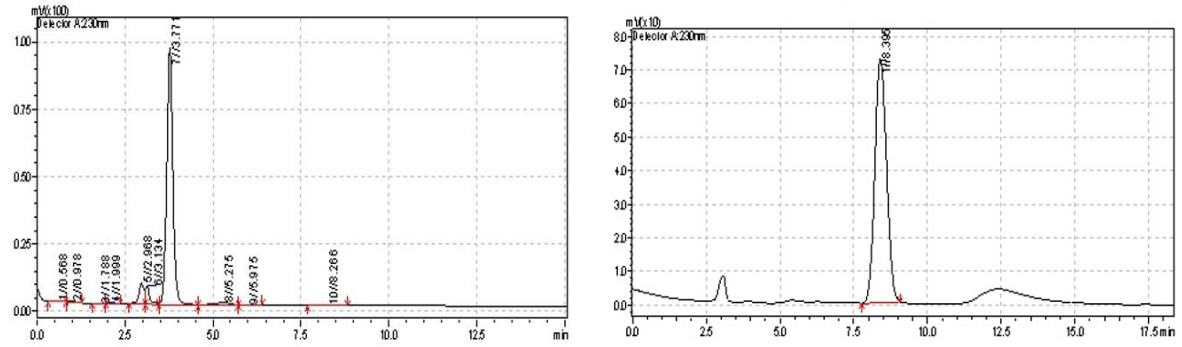


c) d)

Figure 1. a) selection of analytical wavelength, b) chromatogram of AZL and MPL in optimized chromatographic condition, c) chromatogram of metoprolol succinate, and d) chromatogram of azelnidipine

Optimized chromatographic conditions

Initially, a mobile phase consisting of methanol and acetonitrile (50:50 v/v) was tested, but it failed to achieve separation of the two drugs. To enhance separation, the composition was modified to a mixture of methanol and water (70:30 v/v), with O-phosphoric acid added to adjust the pH to 3. The final system operated at a flow rate of 1 mL/min, with 230 nm selected as the detection wavelength.

This optimized chromatographic method successfully achieved distinct separation of AZL and MPL, making it the preferred analytical condition. As shown in **Table 1** and **Figure 1**, the retention times for MPL and AZL were observed at 3.8 minutes and 8.5 minutes, respectively.

Table 1. Optimized chromatographical condition

Parameters	Optimized condition
Stationary phase	Hibar ODS C18 5µ column (250 x 4.6 mm)
Mobile phase (v/v)	Methanol: Water (60:40 v/v) pH adjusted to 3 by using 20% orthophosphoric acid
Flow rate (mL/min)	1 mL/min
Detection wavelength (nm)	230 nm
Temperature	Ambient
Injection volume (µL)	20 µL
Run time (minute)	15 minutes
Retention time (minute)	MPL (3.8 min.) and AZL (8.5 min.)

Evaluation of system suitability

To assess the reliability of the chromatographic system, the selected mixture solution was injected five times. The analysis demonstrated high column efficiency, as indicated by a theoretical plate count exceeding 2000. The tailing factors were determined to be 1.16 for MPL and 0.0065 for AZL, ensuring optimal peak symmetry.

Retention times were recorded as 3.94 ± 0.0078 minutes for MPL and 8.57 ± 0.009 minutes for AZL, with an RSD value below 1 for all parameters. These results confirm that the method meets the necessary system suitability criteria, ensuring precise and reproducible chromatographic performance **(Table 2)**.

Table 2. System suitability parameters

Parameter	MPL	RSD	AZL	RSD
Retention time (R_t)	3.94 ± 0.0078	0.198	8.57 ± 0.009	0.116
Tailing factor	1.16 ± 0.0065	0.561	1.048 ± 0.0094	0.903
Number of theoretical plates	3266 ± 30.97	0.948	2556.8 ± 24.67	0.964
Resolution (R_s)	2.11 ± 0.009		9.69 ± 0.025	

Validation of the developed RP-HPLC method

The proposed RP-HPLC method was validated according to ICH guidelines to ensure its reliability. The method demonstrated linearity within the concentration ranges of 8–40 µg/mL for AZL and 25–125 µg/mL for MPL, with regression coefficients (R^2) of 0.998 for MPL and 0.996 for AZL **(Figure 2)**.

Precision and repeatability

The method exhibited strong repeatability across the tested concentration range. The relative standard deviation (RSD) values for intraday precision were 1.26–1.54% for AZL and 1.40–1.59% for MPL, while interday precision results ranged from 1.70–1.88% for AZL and 1.56–1.92% for MPL. All RSD values remained below 2%, confirming the method's precision.

Robustness and accuracy

The developed approach proved robust, as slight variations in the mobile phase composition, flow rate, and pH did not significantly impact results. Furthermore, assay values were found to be 99.72% for AZL and 99.94% for MPL, ensuring high accuracy **(Table 3)**.

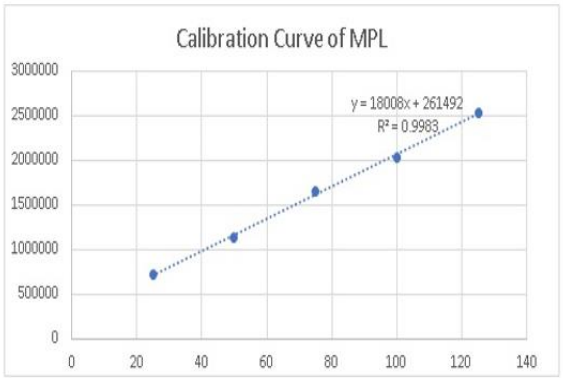
Table 3. Assay data for AZL and MPL

Drug	Amount took (µg.mL^{-1})	Amount found (µg.mL^{-1})	% Assay
AZL	16	15.95 ± 0.06	99.72 ± 0.37
MPL	50	50 ± 0.03	99.94 ± 0.14

A summary of all validation parameters is provided in **Table 4**, demonstrating the method's reliability for routine pharmaceutical analysis.

Table 4. Summary of all validation parameters

Parameter	Limit	Result		Conclusion
		AZL	MPL	
Linearity and range	$R^2 > 0.995$	0.996 (8–40 µg/mL)	0.998 (25–125 µg/mL)	Method was linear
Repeatability	RSD < 2	1.22–1.91	1.64–1.99	Method was repeatable
Intraday precision	RSD < 2	1.26–1.54	1.40–1.59	Method was precise
Inter-day precision	RSD < 2	1.70–1.88	1.56–1.92	Method was precise
% Recovery	98 - 102 %	99.74–100.07 %	99.57–100 %	Method was accurate
Robustness	RSD < ++2	0.221–0.347	0.040–0.110	Method was robust
Assay	98–102 %	99.72 %	99.94 %	Pass



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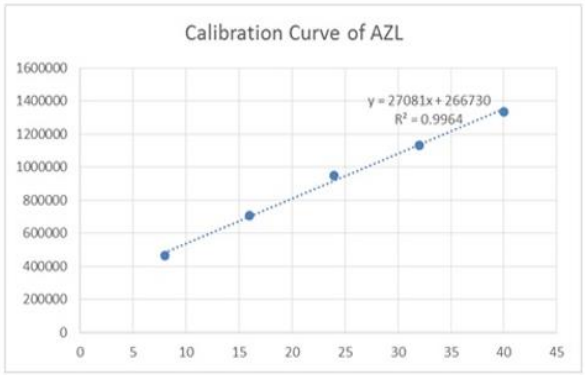

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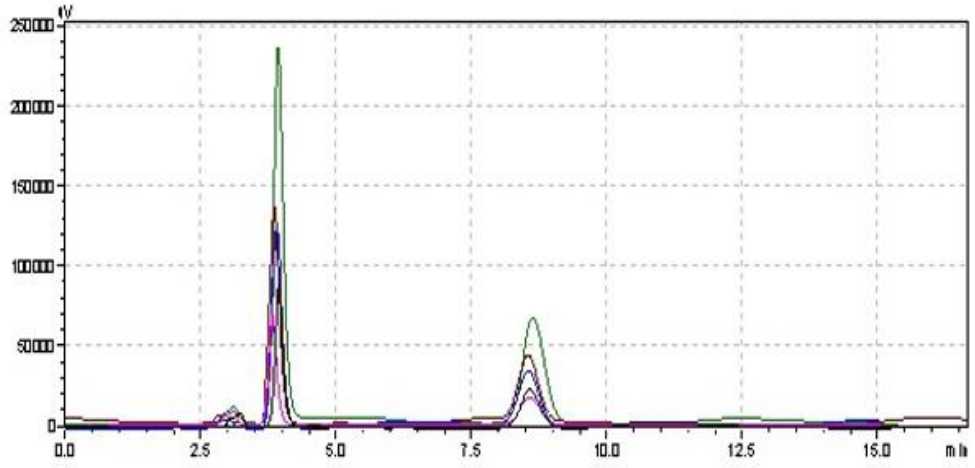

c)

Figure 2. a) calibration curve of MPL, b) calibration curve of AZL, and c) overlain chromatogram for linearity

Conclusion

A reliable RP-HPLC method was successfully developed and validated for the quantification of azelnidipine (AZL) and metoprolol succinate (MPL) in a synthetic mixture. The method adhered to ICH guidelines, ensuring its precision, accuracy, linearity, and robustness.

Since no analytical method has been reported for the simultaneous estimation of AZL and MPL in synthetic mixtures, this validated approach fills a critical gap. With its high precision and compliance with regulatory standards, this method can be effectively utilized for routine quality control and pharmaceutical analysis of these antihypertensive drugs.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Shewale VU, Aher SS, Saudagar RB. Azelnidipine: a review on therapeutic role in hypertension. J Drug Deliv Ther. 2019;9(3-s):1002-5. doi:10.22270/jddt.v9i3-s.3090
2. Papadopoulos DP, Papademetriou V. Metoprolol succinate combination in the treatment of hypertension. Angiology. 2009;60(5):608-13. doi:10.1177/0003319708326450
3. Ghore MG, Dabhade PS. RP-HPLC method development and validation of azelnidipine. Int J Pharm Sci Res. 2016;7(12):5111-4. doi:10.13040/IJPSR.0975-8232
4. Mandale D, Mistry R, Chauhan N. An analytical approach of azelnidipine: a review. World J Pharm Pharm Res. 2021;10(3):682-92. doi:10.20959/wjpps20213-18460
5. 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(2):831-47. doi:10.25004/IJPSDR.2021.130308
6. Kumar M, Chandra U, Garg A, Gupta P. A stability indicating RP-HPLC method validation for simultaneous estimation of azelnidipine and telmisartan in a fixed-dose combination. Int J Pharm Sci Res. 2021;13(3):288-94. doi:10.25004/IJPSDR.2021.130308

7. Chavda N, Kumar S. A review article on analytical method development for the combination of azelnidipine and telmisartan. Asian J Pharm Anal. 2021;11(3):227-34. doi:10.52711/2231-5675.2021.00040
8. Rane AS, Mahajan SK. Validation and forced stability-indicating HPTLC method for determination of Azelnidipine. World J Pharm Res. 2016;5(9):1053-62. doi:10.20959/wjpr20169-6930
9. Suneetha G, Venkateswarlu P. Sensitive analysis of azelnidipine and related derivative in human plasma by ultra-performance liquid chromatography-mass spectrometry. Asian J Chem. 2013;15(18):10319-21. doi:10.14233/ajchem.2013.15290
10. Shashank S, Veerma R, Divya V, Anurag V. Analytical method development and validation of metoprolol succinate by high-performance liquid chromatography and ultraviolet spectroscopy technique. Res J Pharm Tech. 2021;14(2):931-7. doi:10.5958/0974-360X.2021.00166.9
11. Emam AA, Naguib IA, Hassan ES, Abdelaleem EA. Development and validation of RP-HPLC and ecofriendly HPTLC method for simultaneous determination of felodipine and metoprolol succinate and their major metabolite in human spiked plasma. J AOAC Int. 2020;103(4):966-71. doi:10.1093/jaoacint/qsz040
12. Desai D, Vasi N, Dalvadi H, Hinge M, Desai S. HPTLC method development and validation of Cilnidipine and Metoprolol succinate in combined dosage form. Pharm Methods. 2016;7(1):28-34. doi:10.5530/phm.2016.7.5
13. Patel DM, Patel D, Patel A, Sheth A. Method development and validation for simultaneous estimation of benidipine hydrochloride and metoprolol succinate in the tablet. J Drug Deliv Ther. 2019;9(6-s):28-30. doi:10.22270/jddt.v9i6-s.3692
14. Singh B, Patel DK, Ghosh SK. Development of reverse phase HPLC method for simultaneous analysis of metoprolol succinate and hydrochlorothiazide in a tablet formulation. Trop J Pharm Res. 2009;8(6). doi:10.4314/tjpr.v8i6.49401
15. Phale MD, Hamrapurkar PD. Optimization and establishment of a validated stability indicating HPLC method for study of the stress degradation behavior of metoprolol succinate. J AOAC Int. 2010;93(3):911-6. doi:10.1093/jaoac/93.3.911
16. Vora BN, Parmar RR, Nayak PP, Shah DA. Development and validation of the simultaneous UV spectrophotometric method for estimation of metoprolol succinate and olmesartan medoxomil in the tablet dosage form. Pharm Methods. 2012;3(1):47-8. doi:10.4103/2229-4708.97724
17. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. ICH harmonized tripartite guideline: validation of analytical procedures: text and methodology Q2(R1), Current Step 4 version. ICH; 2005.
18. Lalthanpuii K, Kaur J, Saini S, Bhatti K, Nain P. Strengthen the monitoring and reporting of adverse drug reaction at a tertiary teaching hospital. Arch Pharm Pract. 2022;13(1):61-7. doi:10.51847/Zq3HaDzGqf