Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

AN RP-HPLC METHOD PERFORMANCE AND VALIDATION FOR AZELNIDIPINE MEASURMENT AND METOPROLOL SUCCINATE WITHIN A SYNTHETIC MIXTURE

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ARTICLE INFO

Received:
20 Feb 2023
Received in revised form:
24 May 2023
Accepted:
27 May 2023
Available online:
28 Jun 2023

Keywords: Azelnidipin, Metoprolol succinate, RP-HPLC, Analytical validation of a method, ICH guidelines

ABSTRACT

Current research paper describes the RP-HPLC method for the determination of Azelnidipine and Metoprolol Succinate from the synthetic mixture. Azelnidipine is a Calcium channel blocker and Metoprolol Succinate is a Beta blocker. Both drugs are from the Anti-Hypertensive class. The present analytical method was developed on Shimadzu HPLC LC2010 which includes a UV-VIS detector and binary gradient system. Separation of the component was achieved on Hibar ODS C_{18} 5 µ column (250 x 4.6 mm) at ambient temperature in isocratic mode with a mixture of Methanol: water (70:30 v/v) as mobile phase (pH – 3.0) at a flow rate of 1.0 ml/min with UV detection at 230 nm. The method shows a linear response in the range of 8-40 µg/ml of Azelnidipine and 25-125 µg/ml of Metoprolol Succinate. The devised approach was successfully used to determine azelnidipine and metoprolol succinate from a synthetic combination and was verified in following ICH Q2 R1 requirements.

To Cite This Article: Singh A, Rajput A, Kureshi G, Carpenter G, Vashi J. An RP-HPLC Method Performance and Validation for Azelnidipine Measurment and Metoprolol Succinate Within a Synthetic Mixture. Pharmacophore. 2023;14(3):1-6. https://doi.org/10.51847/QC9qqMCNoh

Introduction

Metoprolol succinate and azelnidipine are both anti-hypertensive medications. Azelnidipine (AZL), a calcium channel blocker, prevents vascular smooth muscle transmembrane Ca2+ influx through voltage-dependent channels. It causes a steady drop in blood pressure, slows down the heartbeat, and exhibits persistent hypotensive effects [1]. Clinical significance of metoprolol succinate in patients with cardiovascular disease. It lowers cardiac output because it blocks beta-1 adrenergic receptors. For the treatment of hypertension, this medication is typically used with calcium channel blockers and diuretics [2]. The principal application of azelnidipine and metoprolol succinate is the management of stage 2 hypertension. (CDSCO approved, 7 August 2020) The recommended therapeutic dose for the combination of azelnidipine and metoprolol succinate is 16 mg and 50 mg, respectively. In order to design a procedure, a dose of 16 mg of AZL and 50 mg of MPL was used. Azelnidipine and metoprolol succinate can be determined separately or in combination with other medications using a variety of analytical techniques. Azelnidipine and metoprolol succinate cannot be determined from a synthetic mixture using any analytical approach, according to a thorough study of the literature [3-17]. Additionally, HPLC is more practical than other chromatographic procedures since it is a process that is incredibly quick, efficient, and accurate. So, out of all the chromatographic techniques, HPLC was chosen as the method of choice.

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Materials and Methods

A gift sample of MPL (99.78% pure) was received for research purposes from Dwarkesh Pharmaceuticals Pvt Ltd., Vatva, and AZL (99.80% pure) was purchased from Pure Chem Pvt Ltd., Ankleshwar. Methanol, HPLC water, and O-phosphoric acid were acquired from Rankem Chemicals, Astron Chemicals, and Merck Chemicals, respectively.

Selection of Detection Wavelength
Working standards of metoprolol succinate (10 g/mL) and azelnidipine (10 g/mL) were made using methyl alcohol as the solvent Choose the wavelength. Overlapping UV scans between 200 and 400 nm were performed on them.

Instrumentation and Chromatographic Condition
Working standards of azelnidipine (10 g/mL) and metoprolol succinate (10 g/mL) were created for wavelength selection using methyl alcohol as the solvent. They were overlapped and scanned in the 200 to 400 nm UV range.

Preparation of Standard Stock Solution of AZL and MPL
For the preparation of a standard stock solution of AZL, accurately weigh 10 mg of AZL in A 100 mL volumetric flask and dilute it with methanol up to the mark (100 μg/mL). Take 1 mL from the above solution and further dilute it with mobile phase in a 10 mL volumetric flask (10μg/mL). similarly, 10 mg of MPL was weighed and diluted to 100 ml with methanol (100 μg/ml) and was further diluted with mobile phase to give a final concentration of 10 μg/ml.

Preparation of Standard Stock Solution of a Mixture
Using methanol (160 + 500 g/mL), a stock solution of the mixture was made by diluting 16 mg of AZL and 50 mg of MPL into a 100 mL volumetric flask. Take 1 mL of the previously prepared solution, and further dilute it with mobile phase into a 10 mL volumetric flask that has final concentrations of MPL and AZL of 50 and 16 g/mL each.

System Suitability Parameters
To determine the system appropriateness characteristics, including Retention time (Rt), Tailing factor (T), Resolution (Rs), and Number of theoretical plates, A solution of AZL+MPL (16+50 g/mL) was injected five times. RSD determined the system suitability parameters for the chosen concentration. The suggested approach was verified using ICH guidelines [18] for linearity, accuracy, precision, and limits of detection and quantification.

Linearity and Range
Prepare the master stock solution by precisely weighing 16 mg and 50 mg of AZL and MPL, respectively, in a 10 mL volumetric flask (1600+5000 g/mL), before testing the method's linearity. In a 10 mL volumetric flask, 5 further dilutions of the aforementioned solution were made, ranging in concentration from 8 to 40 g/ml for AZL and 25 to 125 g/ml for MPL. Utilizing ideal chromatographic conditions, all of the aforementioned solutions were injected at a volume of 20 L into the column.

Repeatability
By using optimized chromatographic conditions, prepared standard solutions with concentrations of AZL (8–40 g/mL) and MPL (25–125 g/mL) was injected at a volume of 20 L into a column. Each standard mixture was injected five times, and the peak area was tracked. RSD evaluated the repeatability of each concentration.

Limit of Detection and Limit of Quantitation
Two techniques were used to establish LOD and LOQ: visual inspection and statistical method using repeatability data. LOD and LOQ were determined using the mean of the slope and the standard deviation of the response.

Accuracy
The accuracy test was carried out by injecting a placebo with a standard. The target concentration was 16+50 g/mL for AZL+MPL. Prepare a mixture with a chosen placebo that has been increased by 50%, 100%, and 150%. The mean% recovery was obtained after three analyses of each spiking concentration.

Intraday and Inter- day Precision
Performing intraday and interday precision allowed for the determination of method precision. Mixtures that represent the full range (AZL+MPL = 8+25, 24+75, and 40+125 g/mL) were examined for intraday precision on the same day at various intervals. On several days, the inter-day precision of a mixture that represents the entire range (AZL+MPL = 8+25, 24+75, and 40+125 g/mL) was examined.

Robustness

To accomplish robustness, the following parameters were changed one at a time, and their effects were assessed by contrasting them with the usual dish.
Then mobile phase flow rate was 0.05 mL/min, while the optimal flow rate was 1 mL/min.
Mobile phase pH was less than 0.5; the ideal pH was 3.
Mobile phase composition (5 mL).
RSD was calculated after three determinations for each change.

Assay

There are 50mg of MPL and 16mg of AZL in the current synthetic mixture. Create a solution that is adequately diluted, mixed with a chosen placebo, and has a final concentration of 16 g/mL for AZL and 50 g/mL for MPL.utilizing ideal chromatographic conditions, the same solution was injected into the column three times at a volume of 20 L, and the mean% assay was computed. The current RP-HPLC method was created using the Shimadzu HPLC LC2010, which has a UV-VIS detector and a binary gradient system with a variety of columns. Separation was accomplished using a Hibar ODS C18 5 column (250 x 4.6 mm) in isocratic mode at room temperature with a mobile phase that was a 70:30 v/v mixture of methanol and water (pH - 3.0) with a flow rate of 1.0 mL/min with UV detection at 230 nm.

Results and Discussion

Selection of Analytical Wavelength

Two iso-absorptive points were discovered after the scanned spectra of AZL and MPL were overlapped. 219 nm and 230 nm are the two iso-absorptive points. 230 nm was chosen as the detecting wavelength for determining the AZL and MPL, respectively, from these two iso-absorptive sites **(Figure 1)**.

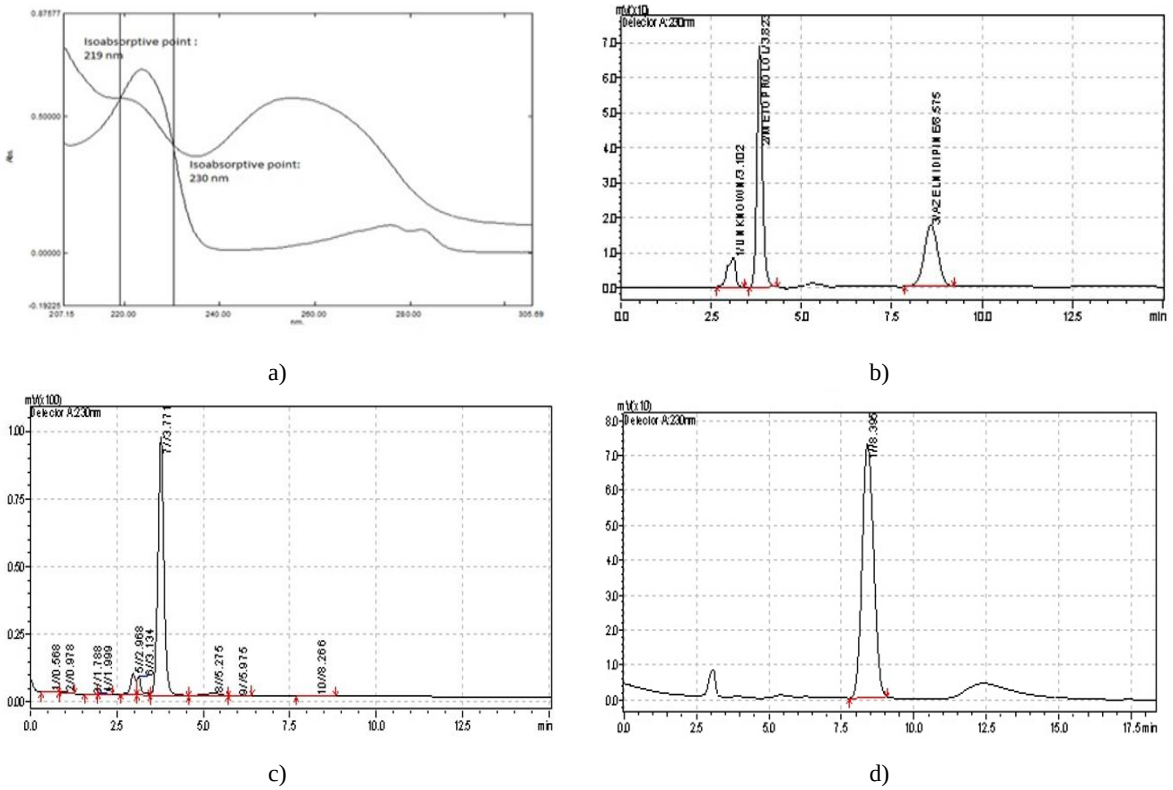


Figure 1. a) selection of analytical wavelength, b) Chromatogram of AZL and MPL in optimized chromatographic condition, c) Chromatogram of Metoprolol Succinate, d) Chromatogram of Azelnidipine

Optimized Chromatographic Condition

Methanol and acetonitrile were used as the mobile phase in the initial study in a ratio of 50:50 by volume, but no separation of the two drugs was noticed. So, water was added to the system along with the methanol in a ratio of 70 parts methanol to 30 parts water (v/v), and O-Phosphoric acid was used to adjust the pH to 3. The system had a flow rate of 1 mL/min, and 230 nm was chosen as the detection wavelength. Since the aforesaid chromatographic technique successfully separated AZL and MPL, it was maintained as the ideal chromatographic condition. According to **Table 1 (Figure 1)**, the observed retention times for MPL and AZL are 3.8 and 8.5 minutes, respectively.

Table 1. optimized chromatographical condition

Parameters	Optimized condition
Stationary Phase	Hibar ODS C_{18} 5µ column (250 x 4.6 mm)
Mobile Phase (v/v)	Methanol: Water (60:40 v/v) pH adjusted to 3 by using 20% Ortho Phosphoric 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.)

System Suitability Parameter

After injecting the chosen mixture solution five times, the system's suitable characteristics demonstrate strong column efficiency with a significant number of theoretical plates (>2000). The measured tailing factors for MPL and AZL were 1.16 and 0.0065, respectively. MPL had a retention time of 3.94 0.0078 and AZL had a retention time of 8.57 0.009 discovered. Each parameter's relative standard deviation was determined. We may state that the system is appropriate because the calculated RSD was less than one **(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 Developed Method

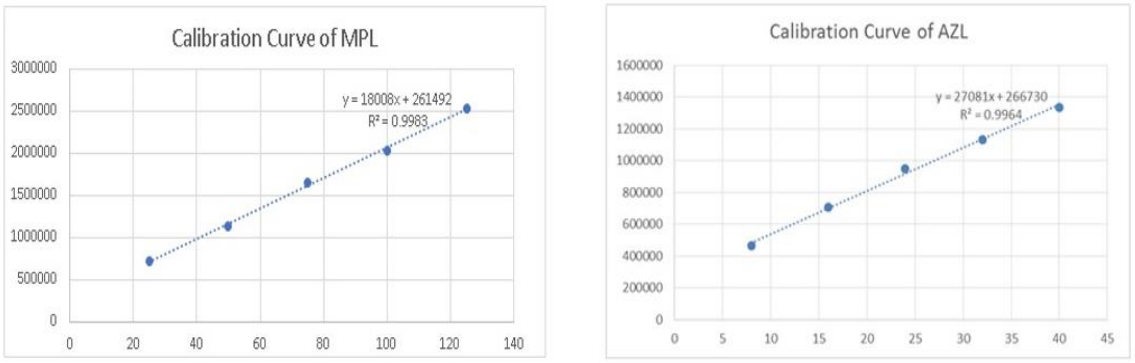
According to ICH guidelines, the developed Reverse Phase HPLC technique has been validated. The devised approach was found to be linear for AZL concentrations of 8–40 g/mL and MPL concentrations of 25–125 g/mL. Following the calibration of the peak area vs. concentration curves for both drugs, the linear regression coefficients for MPL and Azl were 0.998 and 0.996, respectively **(Figure 2)**. The method was proven to be repeatable for AZL over the range of 8–40 g/mL and for MPL over the range of 25–125 g/mL. RSD was computed for each concentration after all the mixes were evaluated at each concentration, and it was discovered to be less than 2. For AZL and MPL, the observed %RSD for intraday precision was 1.26 - 1.54 and 1.40 - 1.59, respectively. For inter-day precision, the observed %RSD ranged from 1.70 to 1.88 for AZL and from 1.56 to 1.92 for MPL. The %RSD value was less than 2, which indicated that the approach was accurate. Because the result did not significantly alter when intentional changes were made to the optimized mobile phase system, the created method was judged to be robust. The % assay for AZL and MPL was found to be 99.72% and 99.94% respectively **(Table 3)**. Summary of all the validation parameters is highlighted in **Table 4**.

Table 3. Assay Data for AZL and MPL

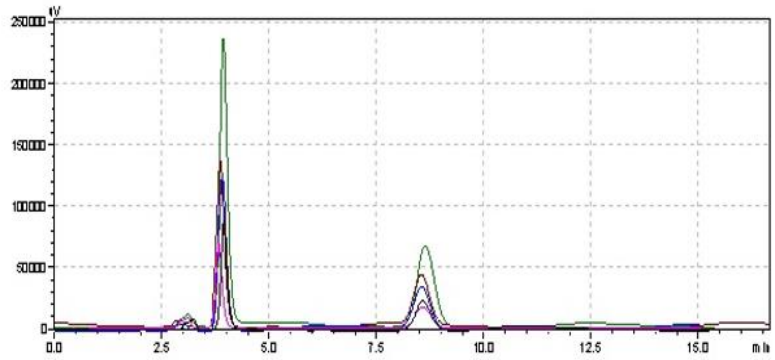
Drug	Amount Took (µg.mL^{-1})	Amount found(µg.mL^{-1})	% Assay
AZL	16	15.95 ± 0.06	99.72 ± 0.37
MPL	50	50 ± 0.03	99.94 ± 0.14

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



a) b)



c)

Figure 2. a) Calibration curve of MPL, b) Calibration curve of AZL, c) Overlain chromatogram for Linearity

Conclusion

A proposed method for determining AZL and MPL from A synthetic mixture was developed, and the developed method was validated following ICH guidelines. The combination of AZL and MPL is currently undergoing a clinical phase III trial. However, there is no analytical method available for determining AZL and MPL from synthetic mixtures. We can infer from the findings that the method devised is precise and adheres to all ICH requirements. In order to identify Azelnidipine and Metoprolol Succinate from a synthetic mixture, the described methods can be used.

Acknowledgments: The samples of AZL and MPL provided by Pure Chem Pvt Ltd. and Dwarkesh Pharmaceuticals Pvt Ltd., without whom this work would not have been possible, are greatly appreciated by the authors. We also wish to express our gratitude to the entire research team for their contributions. The Parul Institute of Pharmacy and Research (Parul University) is also acknowledged by the authors for offering first-rate research facilities and encouraging research endeavors.

Conflict of interest: None

Financial support: Thanks for financial support Dwarkesh pharmaceutical, Rankem Chemical, Astron Chemical, Merck Che,ical,

Ethics statement: This is my original research work.

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