Ind. J. Pharm. Edu. Res., 2025; 59(2s):s680-s688.
https://www.ijper.org

Original Article

Method Development for Estimation of Azelnidipine and S(-)MetoprololSuccinateinTabletsbyUVSpectrophotometry and HPLC Using Student's *t*-Test

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ABSTRACT

Introduction: Azelnidipine a calcium channel blocker belongs to BCS class II and S (-) Metoprolol Succinate belongs to BCS class I. **Rationale:** Calcium is responsible for inducing smooth muscle contraction. Inhibiting calcium channel will inhibit the contraction of smooth muscles which results in reduction of blood pressure. Metoprolol selectively acts on β-1adrenergic receptor (specifically related to cardiac cells). Metoprolol shows negative ionotropic and chronotropic effect. Thus, it shows synergistic or complementary action azelnidipine. **Objectives:** To formulate and evaluate the new combination of antihypertensive drugs and UV and reverse phase chromatography method development for quantification of Azelnidipine and Metoprolol in its formulation. **Materials and Methods:** For the simultaneous estimation of Azelnidipine and S (-) Metoprolol Succinate by UV-spectrophotometry two UV-spectrophotometric methods were developed using Schimadzu-1800 UV spectrophotometer. RP-HPLC method was developed using Inertsil ODS C_{18} (150X4.6 mM; 5 µM) column by flowing solvent system sodium dihydrogen phosphate anhydrous Buffer: Acetonitrile (35:65) at the 1 mL/min. Detection of chromatographic peaks were carried out by employing a 235 nm UV detector. **Results:** According to ICH recommendations, the developed methods were validated. The suggested technique demonstrated appropriateness for concurrent determination of azelnidipine and metoprolol using a UV spectrophotometer and HPLC. **Conclusion:** The method established via UV-Spectrophotometry and HPLC was evaluated to be unique, easy, accurate and exact. The technique can be applied to routine quality control of bulk and combined dose forms of Azelnidipine and S (-) Metoprolol Succinate in pharmaceutical and drug research laboratories.

Keywords: Azelnidipine, Metoprolol, RP-HPLC, UV-Spectrophotometry.

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Received: 07-12-2023;
Revised: 29-06-2024;
Accepted: 17-01-2025.

INTRODUCTION

Azelnidipine, a long-acting calcium channel blocker, is particularly lipid-soluble and targeted for the vascular divider and is relied upon to increasingly affect Cerebral Blood stream (CBF). Chemically it is 3-O-(1-benzhydrylazetidin-3-yl) 5-O-propan-2-yl 2-amino-6-methyl-4-(3- nitrophenyl)-1,4-dihydropyridine-3,5- dicarboxylate (Figure 1).[1-4] Metoprolol is a selective beta-1 blocker that is frequently used in succinate and tartrate derivatives, depending on whether the formulation is intended for immediate release or delayed release.[5] Chemically it is 1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-ylamino) prop-2-ol (Figure 2).[6] The rationale behind this work was calcium is responsible for inducing smooth muscle contraction. Inhibiting calcium channel will inhibit the contraction of smooth muscles which results in reduction of blood pressure. Metoprolol selectively acts on β-1adrenergic receptor (specifically related to cardiac cells). Metoprolol shows negative ionotropic and chronotropic effect. Thus, it shows synergistic or complementary action azelnidipine. The significant objective of this work was to formulate and evaluate the new combination of antihypertensive drugs and UV and reverse phase chromatography method development for quantification of Azelnidipine and Metoprolol in its formulation. There are no reported analytical techniques are available in the literature for this combination. We studied analytical methods for individual drugs to develop the method for combination.

MATERIALS AND METHODS

Chemicals and reagents

Methanol and Acetonitrile was acquired from Loba Chemicals Pvt. Ltd., Mumbai. Sodium dihydrogen phosphate anhydrous (buffer) was acquired from Rankem Chemicals Ltd., Azelnidipine and S (-) Metoprolol Succinate was Kindly Provided as gift

DOI: 10.5530/ijper.20253109

samples by Glenmark Pharmaceuticals Ltd., Nashik and Emcure Pharmaceuticals Ltd., Pune respectively. Combination tablets containing 8 mg of AZEL and 50 mg METO was formulated in laboratory.

Instrumentation

Schimadzu 1800 UV Spectrophotometer with UV probe Software was used. HPLC used for analysis was make of Agilent 1260 infinity equipped with PDA detector and 20 µL loop injector. Data acquisition was carried out using open lab software.

Preparation of working and standard stock solutions

Weigh accurately 25 mg of AZEL and 25 mg of S (-) Metoprolol Succinate in separate 25 mL volumetric flasks. Add 12.5 mL of methanol sonicates it for 15 min. Made the volume to 25 mL with methanol. To make a concentration of 50 µg/mL of AZEL and 50 µg/mL of METO, pipette 5 mL of stock solution into a 100 mL flask and add methanol to make up the volume. 1 mL of the second stock solution was taken out, diluted with methanol to form 10 mL and then the concentration of AZEL and METO was adjusted to 5 µg/mL.

Analytical methods

UV Spectrophotometric methods

Method: I Simultaneous equation method

Working standard solution having concentration 5 µg/mL of AZEL and 5 µg/mL of METO was scanned within 200-400 nm to determine λ_{max} of both drugs. The λ_{max} of AZEL was found 255 nm and 222 nm for METO (Figure 3). The standard solution of both drugs was devised in range of 5-30 µg/mL and their corresponding measurement of absorbance at 255 nm and 222 nm and absorptivity coefficients were calculated using Beer's-Lamberts law.[7] For results of assay by simultaneous equation method refer (Table 1).

$$C_X = \frac{A1ay2 - A2ay1}{ax1ay2 - ax2ay1} \text{ ----------(1)}$$

$$C_Y = \frac{A1ax2 - A2ax1}{ay1ax2 - ay2ax1} \text{ ----------(2)}$$

where,

X and Y is Azelnidipine and S (-) Metoprolol Succinate respectively,

Azelnidipine and S (-) Metoprolol Succinate absorbances at 255 nm and 222 nm are A1 and A2, respectively,

AZEL's absorptivities at 255 nm and 222 nm are aX1 and aX2, respectively,

METO absorptivities at 255 and 222 nm are ay1 and ay2, respectively.

Method-II Q-Absorbance ratio method

Q-Absorbance ratio method used isoabsorptive wavelength and one wavelength used was the wavelength of any one drug. The standard solution of both drugs was produced in range of 5-30 µg/mL and their corresponding measurement of absorbance at 255 nm and 235 nm and absorptivity coefficients were calculated using Beer's-Lamberts law.[8]

$$C_X = \frac{(Qm - Qy)}{(Qx - Qy)} X \frac{A}{ax} \text{ ----------(1)}$$

$$C_Y = \frac{(Qm - Qx)}{(Qy - Qx)} X \frac{A}{ay} \text{ ----------(2)}$$

$Q_m = A_2 / A_1$,

$Q_x = ax_2 / ax_1$,

$Q_y = ay_2 / ay_1$.

where,

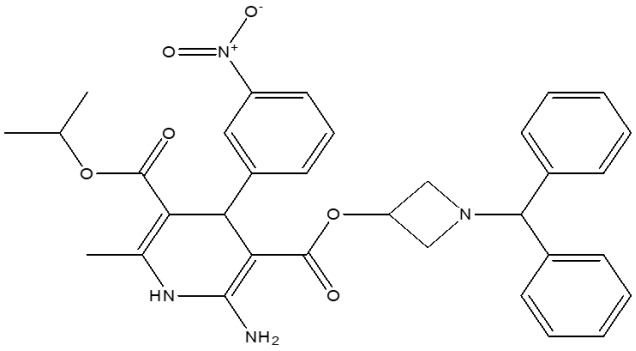

Figure 1: Chemical structure of Azelnidipine.

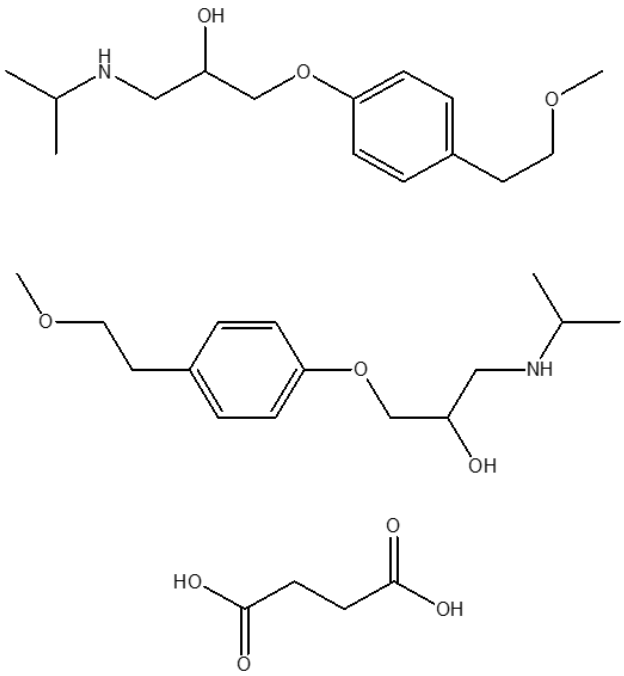

Figure 2: Chemical structure of S (-) Metoprolol Succinate.

Cx, Cy: AZEL and METO concentrations respectively (g/100 mL),

Qx: Absorptivity ratio for AZEL at 255 nm and 235 nm,

Qy: Absorptivity ratio for METO at 255 nm and 235 nm,

Qm: Absorbance ratio for Mixture at 255 nm and 235 nm,

A: Mixture absorbance at Isobestic wavelengths,

ax: Absorptivity of AZEL at Isobestic wavelength,

ay: Absorptivity of METO at Isobestic wavelength.

For the results of assay by absorption ratio method refer (Table 2).

Method-III Derivative spectroscopy

Working standard of solution containing 5 µg/mL of Azelnidipine and S (-) Metoprolol Succinate was scanned in wavelength range 200-400 in derivative mode. The derivative spectrum for first order, second order and third order was recorded (Figure 4). Each sample was scanned three times.[9]

RP-HPLC method

HPLC separation was carried out using Agilent 1260 infinity LC equipped with Inertsil ODS C_{18} column using mobile phase Acetonitrile: Sodium dihydrogen phosphate anhydrous Buffer (65:35). Buffer used for chromatographic separation was Orthophosphoric acid was used to modify the pH of the sodium dihydrogen phosphate buffer to 3.0. 1.0 mL/min of flow was employed for separation for 10 min. 20 µL of the sample injection volume was used. Detection of azelnidipine and metoprolol succinate was achieved at 235 nm using UV detector. The HPLC chromatogram was recorded (Figure 5). Elution time for Azelnidipine 2.6 min and for S (-) Metoprolol Succinate 3.7 min. For results of assay by HPLC method refer (Table 3).

Procedure for analysis of tablet formulation

Combination Tablets of AZEL and METO in 8:50 mg was formulated in laboratory.[10-14]

Table 1: Assay data of formulated tablets by simultaneous equation method.

Drug content	Concentration (µg/mL)	Found mean concentration (µg/mL)	Mean % Assay	%RSD of analytes
Azelnidipine	8	7.95	99.3	0.56
S (-) Metoprolol	50	49.3	98.6	0.85

Table 2: Assay data of formulated tablets by absorbance ratio method.

Drug content	Concentration (µg/mL)	Found mean concentration (µg/mL)	Mean % Assay	%RSD of analytes
Azelnidipine	8	7.92	99.0	0.96
S (-) Metoprolol	50	49.9	99.8	0.65

Table 3: Assay data of formulated tablets by HPLC method.

Formulation	Label Claim		% Assay	
	AZEL (mg)	METO (mg)	AZEL	METO
Tablet	8	50	99.60	99.23

Table 4: Accuracy data of AZEL and METO by simultaneous equation method.

% Accuracy Level (*n*=3)	Desired sample solution	Total amount of standard spiked	Total concentration (µg/mL)	Mean absorbance	Concentration detected (µg/mL)	% RSD of analytes	% Recovery of analytes
AZEL							
50	10	5	15	0.17236	15.1	0.29	100.80
100	10	10	20	0.19116	19.8	0.35	99
150	10	15	25	0.26487	24.97	0.98	99.8
METO							
50	10	5	15	0.13393	14.95	0.38	99.67
100	10	10	20	0.16558	19.93	0.65	99.65
150	10	15	25	0.20454	25	0.99	100

Table 5: Accuracy data of AZEL and METO by Absorbance ratio method.

% Accuracy Level (*n*=3)	Desired sample solution	Total amount of standard spiked	Total concentration (µg/mL)	Mean absorbance	Concentration detected (µg/mL)	% RSD of analytes	% Recovery of analytes
AZEL							
50	10	5	15	0.14352	14.89	0.63	99.27
100	10	10	20	0.18555	19.89	0.93	99
150	10	15	25	0.26393	24.89	0.89	99.2
METO							
50	10	5	15	0.13948	14.77	0.68	98.47
100	10	10	20	0.17101	20.05	0.12	100.25
150	10	15	25	0.19421	24.96	0.79	99.84

Table 6: Summary of validation parameters of simultaneous equation method.

Parameters	AZEL		METO	
	255 nm	222 nm	222 nm	255 nm
Linearity	5-30 µg/mL		5-30 µg/mL	
Correlation coefficient (R^2)	0.9925	0.9979	0.9915	0.9912
Repeatability (%RSD)	0.51	0.89	1.2	0.95
% Recovery	99-100.8		99-100	
% Assay	99.3		98.6	

Table 7: Summary of validation parameters of absorbance ratio method.

Parameters	AZEL		METO	
	255 nm	235 nm	235 nm	255 nm
Linearity	5-30 µg/mL		5-30 µg/mL	
Correlation coefficient (R^2)	0.9976	0.9975	0.9939	0.9961
Repeatability (%RSD)	0.61	0.94	1.02	0.87
% Recovery	99-100.8		99-100	
% Assay	99.0		99.8	

Accurately weigh and crush twenty tablets and transferred powder containing to 50 mg of metoprolol succinate to 100 mL flask add 70 mL of diluent (Mobile Phase), sonication was carried out for 15 min. Made the volume 100 mL. Use a 0.45 µM nylon filter to filter the solution. From filtrate pipette out 5 mL and transferred to the 50 mL of volumetric flask.

RESULTS AND DISCUSSION

As per ICH Q2 (R1) recommendations, the developed UV technique was validated for all validation parameters. RP-HPLC technique was validated for repeatability, robustness and precision. All system suitability parameters were also calculated for HPLC method.[9]

Accuracy

Accuracy for UV method was performed in 50%, 100% and 150% accuracy levels. Accuracy was performed by using standard addition method. Sample prepared in triplicate and % RSD was calculated (Tables 4 and 5).[8,9,15] Accuracy by UV spectrophotometry was found within range of 98-102%.

Table 8: Precision for developed method by HPLC.

Sample no.	Peak Area for azelnidipine	Peak area for S (-) Metoprolol Succinate
1	34852	74213
2	33256	74521
3	35225	74856
4	35452	75886
5	35624	73985
6	33215	74214
% RSD	1.25	1.58

Table 9: System suitability by HPLC.

Parameter	Azelnidipine	S (-) Metoprolol Succinate
Peak Area	35660	74521
Theoretical plate count	4523	3256
Tailing Factor	1.05	1.08
Resolution	-	5.26
Retention Time	2.6 min	3.7 min

Table 10: Robustness for developed method by HPLC.

Change in wavelength (±2 nm)	Azelnidipine peak area	S (-) Metoprolol Succinate peak area	%RSD
233	34550	74559	0.25
235	33965	73995	0.95
237	33654	75965	1.09

Table 11: Linearity results for derivative spectroscopy.

Statistical parameters	UV derivative spectroscopy					
	First order		Second order		Third order	
	Azelnidipine	S(-) Metoprolol	Azelnidipine	S(-) Metoprolol	Azelnidipine	S(-) Metoprolol
Linearity range of analytes	5-30 µg/mL	5-30 µg/mL	5-30 µg/mL	5-30 µg/mL	5-30 µg/mL	5-30 µg/mL
Regrassion coefficient (R^2)	0.999	0.9984	0.9991	0.9977	0.9972	0.9983

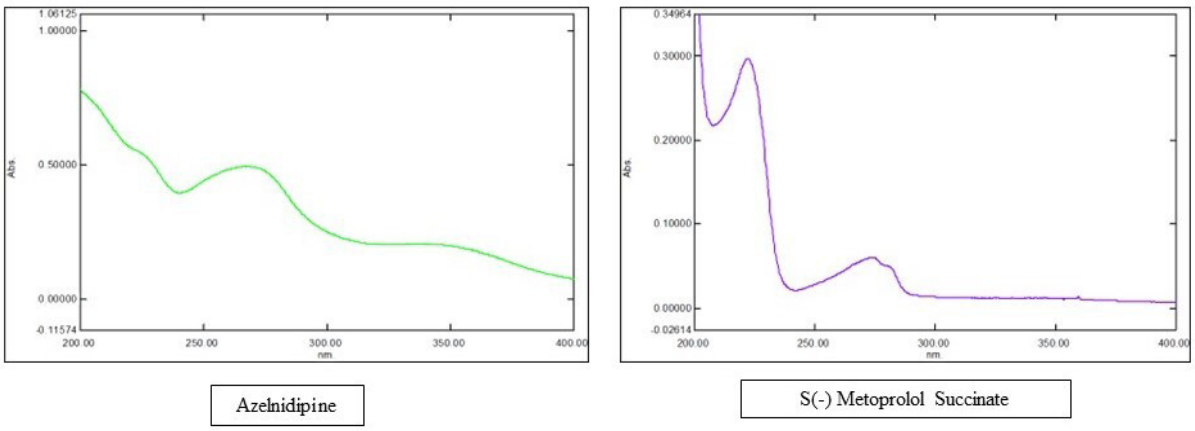

Figure 3: Peak purity Spectra for Azelnidipine and S(-) Metoprolol succinate.

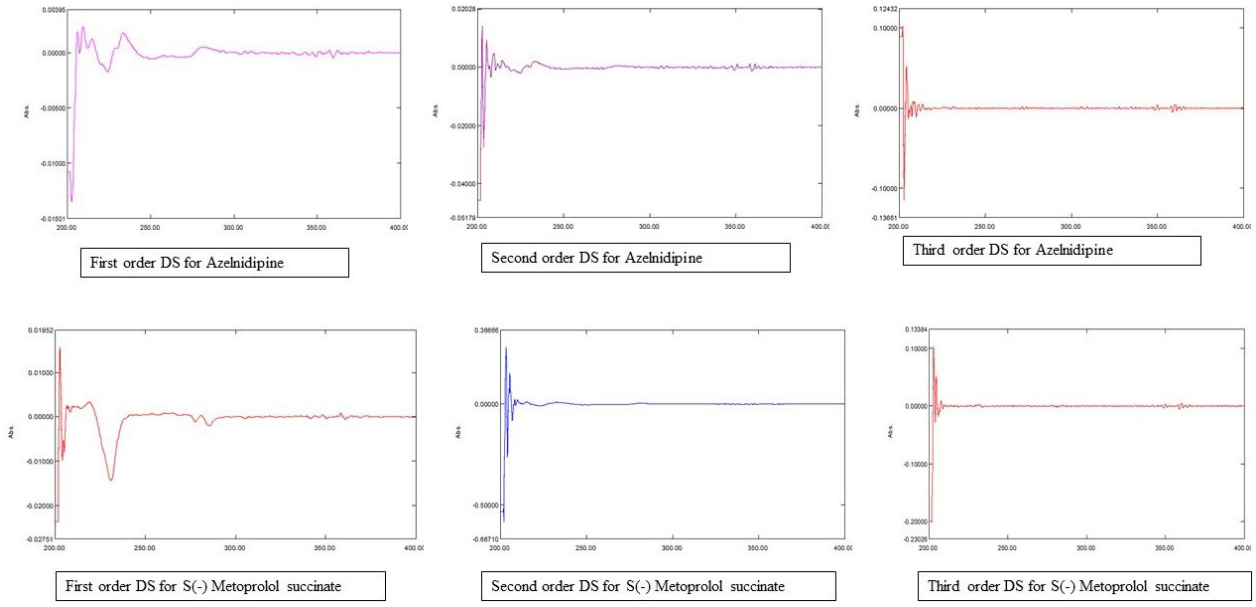

Figure 4: Derivative spectrum for the Azelnidipine and S(-) Metoprolol succinate.

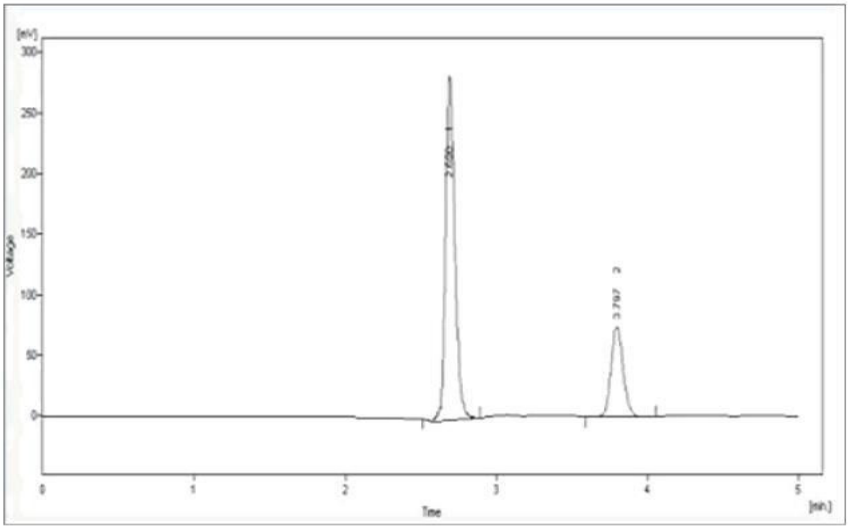

Figure 5: Chromatogram for system suitability.

Table 12 : Application of Student's *t*-test.

Student's t-test for both methods	*p*-value for analytes	
	AZEL	METO
First order DS and HPLC	0.001	0.0015
Second order DS and HPLC	0.0025	0.002
Third order DS and HPLC	0.0045	0.0021

Precision

Precision was performed by preparing three replicates of sample solution and scanned at defined wavelength for both drugs. Precision for HPLC was carried out by performing repeatability of method. % RSD was calculated for both UV methods and HPLC method (Tables 6-8).[8,9,15] The % RSD for both methods were found below 2% which is within acceptance criteria as per ICH guideline Q2 (R1).

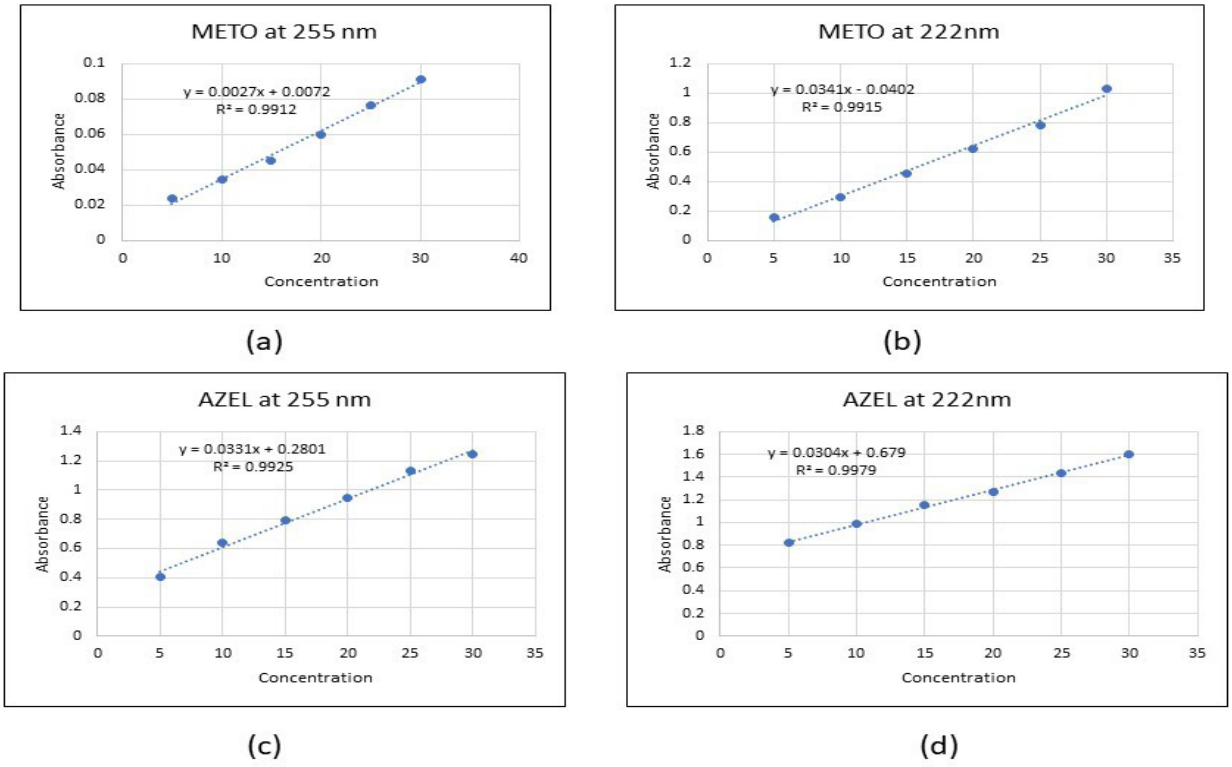

Figure 6: Calibration curve for AZEL and METO by simultaneous equation method. (a) Linearity curve for METO at 255 nm (5-30 ppm), (b) Linearity curve for METO at 222 nm (5-30 ppm), (c) Linearity curve for AZEL at 255 nm (5-30 ppm), (d) Linearity curve for AZEL at 222 nm (5-3 ppm).

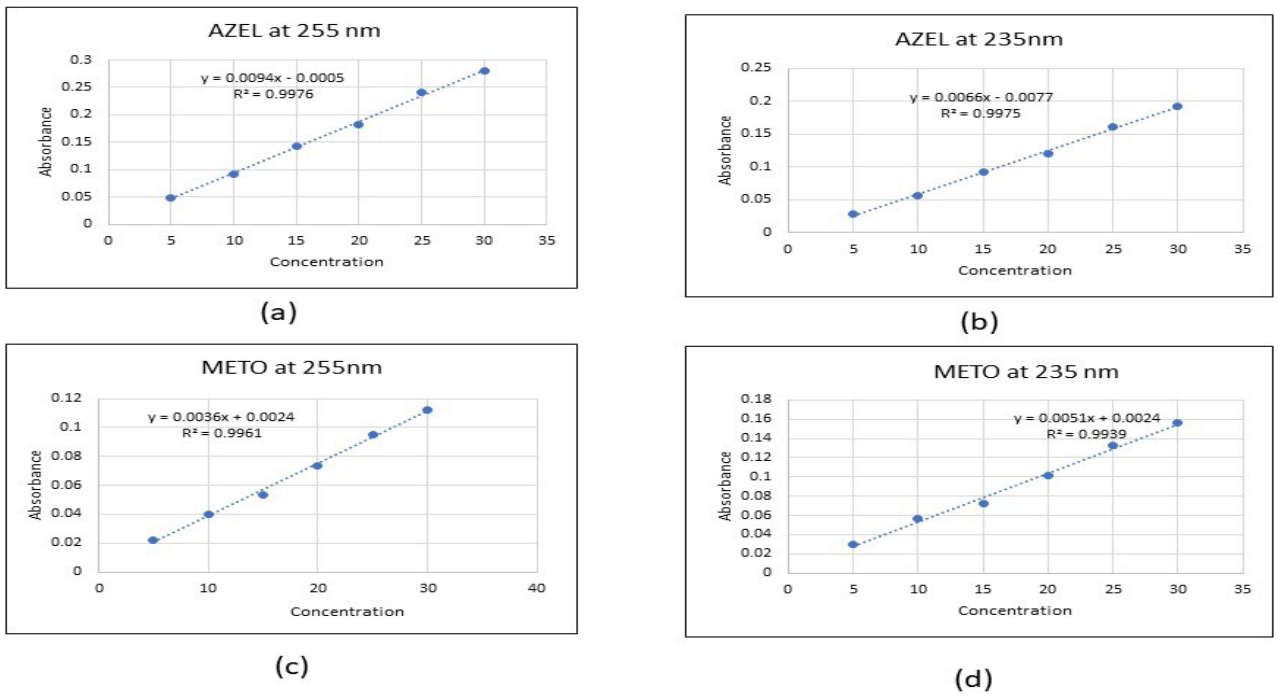

Figure 7: Calibration curve for AZEL and METO by absorbance ratio method. (a) Linearity curve for AZELat 255 nm (5-30 µg/mL), (b) Linearity curve for AZELat 235 nm (5-30 ppm), (c) Linearity curve for METO at 255 nm (5-30 ppm), (d) Linearity curve for METO at 235 nm (5-30 µg/mL).

System suitability

It is performed by injecting five replicates of mixed standard solution and chromatograms were recorded. Theoritical plate count, taling factor, resolution were calculated (Table 9).

Robustness

By purposefully modulating the wavelength and flow rate of the developed approach, robustness was achieved. Chromatograms were taken and the HPLC method's% RSD was calculated (Table 10).[8,9,15] The % RSD for both methods were found below 2% which is within acceptance criteria as per ICH guideline Q2 (R1).

Linearity

The calibration curve was plotted for both drugs. Calibration curve shows good linearity in concentration range 5-30 µg/mL for UV spectrophotometry (Figures 6 and 7). The solution was scanned at defined wavelength for both drugs. The linearity was determined by calculating regression coefficient and slope.[10] the regression coefficient obtained was by simultaneous equation method and absorption ratio method was above 0.99 which is within acceptance limit (Table 11).

CONCLUSION

For the estimation of both pharmaceuticals in tablet form and in bulk, the UV spectrophotometric approach was developed. The developed technique was evaluated and found to be reliable, linear and precise. With a significantly shorter retention time of 2.61 min for azelnidipine and 3.7 min for S (-) Metoprolol Succinate and a tailing factor of 1.02 for azelnidipine and 1.1 for S (-) Metoprolol Succinate, an innovative, easy, accurate, robust and reliable approach was discovered to have been developed by HPLC. This shows that the peak was well within accepted limits. According to the student's T-test, both methods provide statistically significant results. The combination dosage form of azelnidipine and S (-) metoprolol may be successfully quantified using the established RP-HPLC method by assay, purity, stability, solubility and *in vitro* dissolution studies. By using Student's t-test to compare the two approaches, it was discovered that the suggested approaches can be employed for the regular analysis of Azelnidipine and S (-) Metoprolol Succinate in mixed dosage forms (Table 12). Overall, it was established that the technique was less time-consuming, linear, accurate, exact, particular, sensitive and robust. In drug testing laboratories and the pharmaceutical industry, the method can be used to execute routine quality control of the combined dosage form of azelnidipine and S (-) Metoprolol Succinate.

ACKNOWLEDGEMENT

The authors are grateful to Glenmark Pharmaceutical Ltd., Nashik and Emcure Pharmaceutical Ltd., Bhosari, Pune for providing gift samples of Azelnidipine and S (-) Metoprolol Succinate respectively. Authors are also appreciating laboratory facilities provided by Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune, Maharashtra, India for this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AZEL: Azelnidipine; **METO:** S (-) Metoprolol Succinate; **HPLC:** High Performance Liquid Chromatography; **ICH:** International Council for Harmonization.

SUMMARY

Azelnidipine and metoprolol were simultaneously measured by UV spectrophotometry using two methods: the simultaneous equation approach and the absorbance ratio method. The developed UV method validated for accuracy, precision linearity as per ICH guidelines. Correlation coefficient for each method was found between 0.99-0.9999. % RSD for both methods were found <2%. HPLC method development was achieved using Inertsil C_{18} column flowing mobile phase Acetonitrile: Buffer (65:35) at flow rate of 1mL/min. Precision of RP-HPLC method was found <2%. Assay of formulated tablets were performed by both UV and HPLC method. The assay of tablets was found 99-100%. The developed method revealed novelty, repeatability and requires less time. The developed technique can be applied to standard laboratory quality control analysis. By using Student's t-test to compare the two approaches, it was discovered that the suggested approaches can be employed for the regular analysis of Azelnidipine and S (-) Metoprolol Succinate in mixed dosage forms.

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Cite this article: Ganorkar S, Bhavsar A, Khiste R. Method Development for Estimation of Azelnidipine and S (-) Metoprolol Succinate in Tablets by UV Spectrophotometry and HPLC Using Student's *t*-Test. Indian J of Pharmaceutical Education and Research. 2025;59(2s):s680-s688.