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DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND METOPROLOL SUCCINATE IN THEIR DOSAGE FORM

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ABSTRACT

A Simple, precise and accurate spectrophotometric method has been developed for simultaneous estimation of Azelnidipine (AZL) and Metoprolol Succinate (MET) in pharmaceutical dosage form. This method was based on UV-spectrophotometric determination of two drugs, using absorbance correction method. It involves measurement of absorbances at two wavelengths 313nm (λ_{max} of AZL) and 275.40nm (λ_{max} of MET) in methanol. Linearity was observed in the concentration range of 5 – 25 µg/ml and 25 - 125 µg/ml for AZL and MET respectively. The accuracy and precision of the method was determined and validated. The method showed good reproducibility and recovery with % RSD less than 2. Method can be successfully applied for the routine analysis of AZL and MET in combined dosage form without any interference by the excipients. The method was validated as per ICH Q2 (R1) guideline and found to be accurately in estimating the drug substances in tablet dosage form.

KEYWORDS: Azelnidipine, Metoprolol Succinate, Absorbance correction method, Method Validation.

INTRODUCTION

Azelnidipine (AZL) (3-[1-(diphenylmethyl)azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5- dicarboxylate) **(Figure 1)** is a new dihydropyridine derivative with calcium antagonistic activity. Azelnidipine is Ca+2 channel blocker inhibits trans membrane Ca +2 influx through the voltage dependent channels of smooth muscle in vascular walls. Normally, calcium induces smooth muscle contraction, contributing to hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of vascular smooth muscle walls and decreased blood pressure.[1]

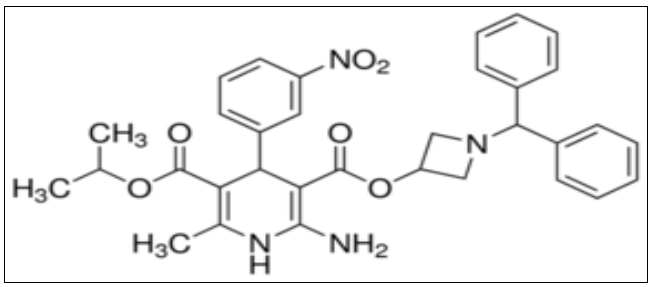

Figure 1: Azelnidipine.[2]

Metoprolol succinate (MET), chemically 1-[4-(- methoxyethyl)phenoxy]-3-(propan-ylamino)propan-2-ol **(Figure 2)** which is used as cardiovascular drug. MET is a β 1- selective (cardio selective) adrenergic receptor blocker.[3] It has the potency to increase the heart rate and decrease renin release from kidney and used in hypotension.[4]

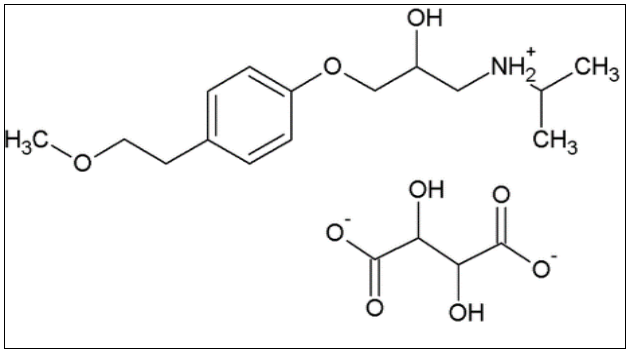

Figure 2: Metoprolol succinate.[5]

This combination of AZL and MET has been introduced for the management of stage-2 hypertension.[6] Both the drugs are used in the ratio of 1:5 (AZL: MET) as Tablet formulation.

Objective of study

On thorough survey of literature, it was revealed that numbers of method have been reported in literature for the individual analysis of AZL and MET by various analytical methods.[7-28] However, no method has been reported for simultaneous estimation of AZL and MET.

So, the present work was aimed to develop a spectroscopic method for simultaneous estimation of AZL and MET and validation the developed analytical method and then prove the utilization of the method for estimation of the combination.

MATERIALS AND METHOD

Reagents and Chemicals

Reference standard of AZL was procured from Purechem Pvt Ltd, Ankleshwar and MET was procured from CTX Lifescience Pvt. Ltd., Surat. Methanol (AR grade) was used as solvent in this method. All the glass wares were calibrated before using.

Instrumentation

A PC enabled (software: UV Probe) UV-Visible double beam Spectrophotometer (Make: SHIMADZU, Model: 2450 and 1800, Japan) with a pair of 1cm matched quartz cells.

Sample and Standard preparation

AZL (10 mg) and MET (50mg) were weighed accurately and transferred to 25 ml volumetric flask, dissolved and volume was made up to mark with Methanol to prepare 100 µg/ml and 500 µg/ml solution of AZL and MET, respectively. The standard solution was subsequently diluted to prepare different concentrations 5-25 µg/ml and 25-125 µg/ml of AZL and MET respectively.

Method validation

The method was validated for limits of detection and quantification, accuracy, precision, assay**.** The method validation was performed according to the recommended guidelines of International Conference Harmonization (ICH).[29]

Linearity

Linearity of the proposed method was assessed by scanning concentrations at five equidistance levels in triplicate. In order to accomplish these working solutions of increasing concentration in the range of 5-25 µg/ml and 25-125 µg/ml of AZL and MET, respectively were prepared from the stock solution of AZL (100 µg/ml) and MET (500 µg/ml). A graph of

Concentration vs. Absorbance was plotted and regression equation was obtained. **(Figure 6 and 7).** Results of linearity were summarized in **Table 3.**

Precision

Repeatability of the method was checked by analysing six different solutions of same concentration (10 µg/ml of AZL and 50 µg/ml of MET) prepared from single stock solution were analysed. **(Table 4)**

Intraday precision performed by evaluating three concentration levels i.e. 5, 10, 15 µg/ml of AZL and 25, 50, 75 µg/ml in case of MET on same day. (**Table 5**)

Interday precision was performed by considering three concentration levels (same as intraday precision) on three different days. All solutions were prepared form different stocks prepared on different days. (**Table 6)**

Accuracy (Recovery study)

It was determined by calculating the recovery of AZL and MET by standard addition method. Accuracy was done by adding both API standard solution and test solution. Total concentration was as per **Table 1.**

Table 1: Solutions for accuracy study.

Concentration of Formulation (µg/ml)		Concentration of API in spiking solution (µg/ml)		Total concentration of (µg/ml)	
AZL	**MET**	**AZL**	**MET**	**AZL**	**MET**
10	50	8	40	18	90
10	50	10	50	20	100
10	50	12	60	22	110

Limit of Detection and Limit of Quantification (LOD and LOQ)

LOD and LOQ were calculated by utilizing data from linearity studies. The mean of slope and standard deviation of intercept of all calibration curves of linearity study was considered for calculating LOD and LOQ as per following formula. The results of LOD and LOQ are summarised in Error! Reference source not found..

$$\textbf{LOD} = 3.3 * \frac{\sigma}{S}$$

$$\textbf{LOQ} = 10 * \frac{\sigma}{S}$$

Where,

σ = Standard deviation of intercept of calibration curve

S = Mean slope of calibration curve

Assay

- All the excipients as per **Table 2** were mixed in 100ml volumetric flask and add 25 ml of methanol then sonicated for 15min. After sonification make up the volume up to 100 ml with methanol. The solution was filtered through Whatman filter paper No. 42.
- Finally the solution had concentration 100µg/ml for Azelnidipine and 500µg/ml for Metoprolol Succinate.

From that pipette out 1.0 ml in 10 ml volumetric flask and volume was made up to mark with methanol to obtain final solution containing 10µg/ml of AZL and 50µg/ml of MET. A zero order derivative spectrum of the resulting solution was recorded and absorbances at 313nm and 275.40nm were noted for estimation of AZL and MET, respectively. The concentrations of AZL and MET in formulation were determined using the corresponding calibration graph.

Table 2: For preparation of tablet.

Ingredients	Quantity taken (mg)
Azelnidipine	10
Metoprolol succinate	50
HPMC	100
Gaur gum	8
SiO_2	12

Robustness

Robustness of the method was determined by subjecting the method to slight change in the method condition, individually, the: Change in Wavelength (± 0.2 nm) and Change in instrument.

RESULTS AND DISCUSSION

Absorbance correction method, the utility of dual wavelength data processing program is its ability to calculate unknown concentration of component of interest in a mixture containing an interfering component. For elimination of the effects of an interfering component, two specific wavelengths were chosen.[30-31]

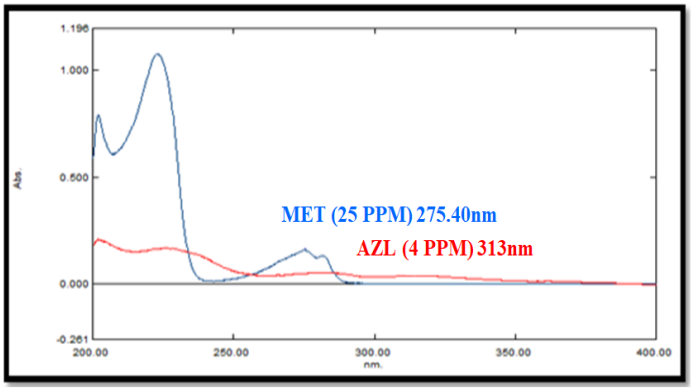

Figure 3: Overlain zero order spectra of AZL and MET.

Selection of wavelength

From spectra at 313nm (λmax of Azelnidipine) Metoprolol Succinate shows zero absorbance so Azelnidipine is directly estimate at 313nm. At 275.40nm (λmax of Metoprolol Succinate) both drugs show some absorbance so Metoprolol Succinate is estimated at 275.40nm using absorbance correction method. (**Figure 4**)

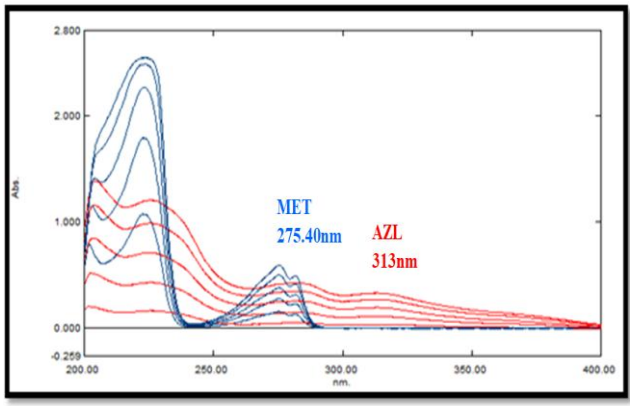

Figure 4: Overlain zero order spectra of AZL and MET.

Linearity

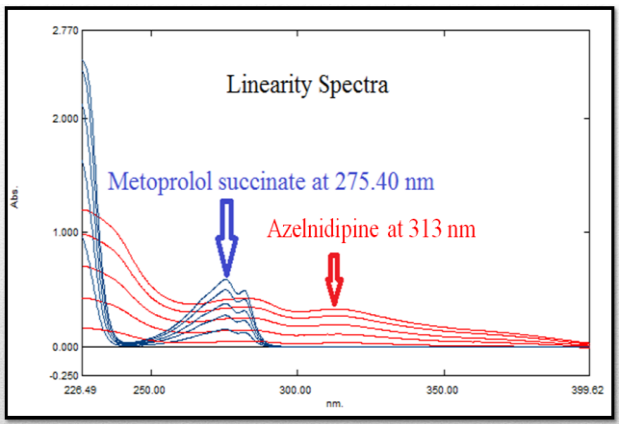

Figure 1: Linearity spectra of AZL and MET.

Table 3: Calibration curve of AZL and MET *(n=3).

Sr. No	Concentration (µg/ml)		Absorbance* (313nm)± SD AZL	Absorbance* (275. 40nm) ± SD MET
	AZL	MET		
1	5	25	0.038 ± 0.0023	0.156 ± 0.00103
2	10	50	0.115 ± 0.0007	0.267 ± 0.00154
3	15	75	0.193 ± 0.0005	0.382 ± 0.00116
4	20	100	0.265 ± 0.0013	0.493 ± 0.00137
5	25	125	0.341 ± 0.0013	0.590 ± 0.00116

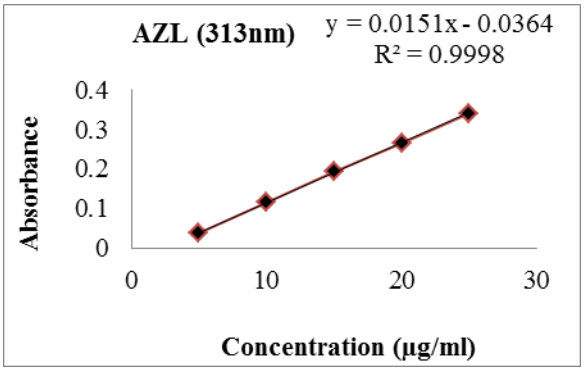

Figure 2: Calibration curve of AZL.

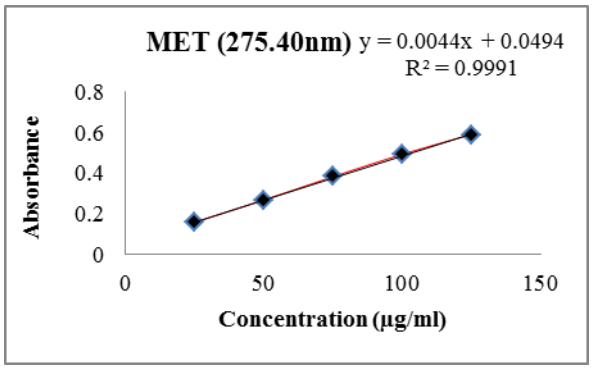

Figure 3: Calibration curve of MET.

Precision

Repeatability

Table 4: Repeatability of AZL and MET *(n=6).

Concentration (µg/ml)		Mean Abs* ± SD		% RSD	
AZL	MET	AZL	MET	AZL	MET
10	50	0.119 ± 0.0014	0.275 ± 0.0010	1.19	0.38

Intraday precision

Table 5: Intraday precision data for estimation of AZL and MET *(n=3).

Conc. (µg/ml)		Abs.* (AZL) Avg. ± SD (313nm)	% RSD	Abs.* (MET) Avg. ±SD (275.40nm)	% RSD
AZL	MET				
5	25	0.038 ± 0.0025	0.64	0.157 ± 0.0015	0.71
10	50	0.117 ± 0.0010	0.54	0.272 ± 0.0010	0.60
15	75	0.191 ± 0.0015	0.46	0.386 ± 0.0020	0.42

Interday precision

Table 6: Interday precision data for estimation of AZL and MET *(n=3).

Conc. (µg/ml)		Abs.* (AZL) Avg. ± SD (313nm)	% RSD	Abs.* (MET) Avg. ±SD (275.40nm)	% RSD
AZL	MET				
5	25	0.040 ± 0.0015	0.65	0.160 ± 0.0010	0.75

10	50	0.118 ± 0.0025	0.49	0.272 ± 0.0011	0.54
15	75	0.192 ± 0.0011	0.40	0.388 ± 0.0015	0.22

Accuracy

Table 7: Recovery data of AZL *(n=3).

Conc. of AZL from formulation (µg/ml)	Amount of Std. AZL added (µg/ml)	Total amount of AZL (µg/ml)	Total amount of AZL found (µg/ml)* Mean ± SD	% RSD AZL	% Recovery (n=3)	% RSD AZL
10	0	10	10.05 ± 0.012	0.26	100.50 ± 0.010	0.30
10	8	18	18.11 ± 0.030	0.16	100.61 ± 0.019	0.19
10	10	20	20.15 ± 0.040	0.19	100.68 ± 0.023	0.22
10	12	22	22.17 ± 0.030	0.13	100.77 ± 0.015	0.15

Table 8: Recovery data of MET *(n=3).

Conc. of MET from formulation (µg/ml)	Amount of Std. MET added (µg/ml)	Total amount of MET (µg/ml)	Total amount of MET found (µg/ml)* Mean ± SD	% RSD MET	% Recovery (n=3)	% RSD MET
50	0	50	50.15 ± 0.025	0.30	100.30 ± 0.035	0.25
50	40	90	90.19 ± 0.015	0.11	100.46 ± 0.012	0.17
50	50	100	100.29 ± 0.015	0.13	100.29 ± 0.023	0.15
50	60	110	110.47 ± 0.016	0.39	100.78 ± 0.017	0.13

LOD and LOQ

Table 9: LOD and LOQ data of AZL and MET *(n=10).

Parameter	AZL (µg/ml) *	MET (µg/ml) *
LOD	0.038	0.066
LOQ	0.115	0.200

Robustness

Table 10: (change in instrument) data of AZL and MET *(n=3).

Condition	Conc. (µg/ml)	Different Instrument			
		Instrument 1	% RSD	Instrument 2	% RSD
Azelnidipine (Mean abs. ±SD)*	05	0.039 ± 0.0015	0.71	0.038 ± 0.0017	0.33
	10	0.117 ± 0.0005	0.32	0.118 ± 0.0011	0.43

(313nm)	15	0.191 ± 0.0010	0.41	0.193 ± 0.0015	0.29
Metoprolol Succinate (Mean abs. ±SD)* (275.40nm)	25	0.159 ± 0.0015	0.41	0.158 ± 0.0020	0.59
	50	0.272 ± 0.0011	0.39	0.273 ± 0.0022	0.38
	75	0.388 ± 0.0020	0.52	0.389 ± 0.0011	0.25

Table 11: (Change in wavelength) data of AZL and MET *(n=3).

Condition	**Conc. (µg/ml)**	**λ_{max} (± 0.2nm)**			
		λ_{max} (312.80nm)	**% RSD**	**λ_{max} (313.20nm)**	**% RSD**
Azelnidipine (Mean abs. ±SD)*	05	0.037± 0.0005	0.37	0.039 ± 0.0015	0.45
	10	0.116 ± 0.0010	0.32	0.117 ± 0.0011	0.51
	15	0.191 ± 0.0011	0.42	0.192 ± 0.0020	0.75
		λ_{max} (275.20nm)	**% RSD**	**λ_{max} (275.60nm)**	**% RSD**
Metoprolol Succinate (Mean abs. ±SD)*	25	0.157 ± 0.0010	0.22	0.160 ± 0.0015	0.65
	50	0.271 ± 0.0020	0.38	0.274 ± 0.0011	0.22
	75	0.387 ± 0.0005	0.47	0.389 ± 0.0010	0.17

Assay

Table 12: Analysis data of synthetic mixture *(n=3).

Sr. No	**Drug**	**Formulation (µg/ml)**	**% Assay* ± SD**	**% RSD**
1	AZL	10	100.50 ± 0.0010	0.30
2	MET	50	100.30 ± 0.0005	0.25

Table 13: Summary of validation parameters.

Parameters	**Absorbance correction method**	
	Azelnidipine	**Metoprolol Succinate**
Concentration range (µg/ml)	5-25	25-125
Regression equation	y= 0.015x – 0.0338	y= 0.0044x + 0.0525
Correlation Coefficient (r2)	0.9998	0.9991
Repeatability (% RSD)	1.19	0.38
Intra-day Precision (% RSD)	0.46 - 0.64	0.42 - 0.71
Inter-day precision (% RSD)	0.40 - 0.65	0.22 - 0.75
Accuracy (% Recovery)	100.50 - 100.77	100.30 - 100.78
LOD (µg/ml)	0.038	0.066
LOQ(µg/ml)	0.115	0.200
Robustness	0.29 - 0.75	0.17 - 0.65
% Assay	100.50	100.30

CONCLUSION

The developed absorbance correction method is simple, precise, specific, and accurate. It is proved that the method was repeatable and selective for the simultaneous estimation of AZL and MET in pure and pharmaceutical dosage forms without any interference from the excipients. This new simple method can be used routinely for the estimation of these drugs.

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