Research Article

Development And Validation Of UV Method For Simultaneous Estimation Of Metoprolol Succinate And Azelnidipine In Pharmaceutical Dosage Form

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ABSTRACT

The UV method for the estimation of Metoprolol and Azelnidipine in bulk and pharmaceutical dosage Form was developed. The identification and quantification was carried out by using UV-VIS spectrophotometer. 0.1% Perchloric acid and Acetonitrile [50:50] used as Diluent for sample and standard preparation and also as a Blank. The wavelength of drug Metoprolol and Azelnidipine were found to be 224 nm and 260 nm respectively. LOD, LOQ values obtained from regression equations of metoprolol and azelnidipine were 1.23, 3.72, 0.31 and 0.94μg/ml. % Accuracy was obtained as 99.94 and 99.85 for Metoprolol and Azelnidipine respectively. %Recovery was obtained as 99.101 and 99.101 respectively. % RSD of the metoprolol and azelnidipine were and found to be 0.26 and 0.57 respectively. attempt has been made to develop UV and Reverse Phase High Performance Liquid Chromatographic method for the estimation of Metoprolol and Azelnidipine in bulk and pharmaceutical dosage form and to validate the developed method according to ICH Q2 (R1) guidelines. Analytical method development was started with the preliminary studies of the drug Metoprolol and Azelnidipine

INTRODUCTION

Azelnidipine is a calcium channel blocker that belongs to the dihydropyridine class. Japan's Daiichi-Sankyo Pharmaceuticals, Inc. is the company responsible for marketing it. When compared to other calcium channel blockers, it has a delayed onset of action and generates a long-lasting drop in blood pressure. Patients who have hypertension can have a progressive reduction in their blood pressure when they take azelnidipine, which is a vasodilator. Because of its vasodilatory effects, azelnidipine does not cause reflex tachycardia, in contrast to other drugs that belong to the same medication class. Metoprolol is a

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cardioselective beta-1-adrenergic receptor inhibitor that inhibits beta1 receptors in a competitive manner while having limited or no effects on beta-2 receptors when administered orally to humans in dosages of less than 100 mg. Through its negative inotropic and chronotropic actions, it brings to a reduction in cardiac output.

MATERIAL AND METHODS:

1. Method Parameters:

a. Diluent: 0.1% Perchloric acid : Acetonitrile (50: 50%, v/v)

Preparation of 0.1% Perchloric acid:

Add 0.1 ml of Perchloric acid in 100 ml of Water, Mix and filtered.

b. Wavelength: $\lambda 1 = 224$ nm; $\lambda 2 = 260$ nm

2. Standard Preparation:

a. Metoprolol Standard Stock Solution-I (MSSS-I):

i. Initially Prepare a Standard Stock Solution (MSSS-I) of by adding 25 mg of Metoprolol in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Metoprolol = 2500 µg/ml).

b. Azelnidipine Standard Stock Solution-II (ASSS-II):

i. Then prepare a Standard Stock Solution (ASSS-II) of Azelnidipine by adding 8 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Azelnidipine = 800 µg/ml).

c. Then add 0.1 ml of MSSS-I &0.1 ml ASSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Metoprolol= 25 µg/ml &Azelnidipine = 8 µg/ml).

3. Selection of Wavelength:

25µg/ml of MET Working Standard and 8µg/ml of AZD Working Standardwere scanned in the UV range of 190-400 nm. The overlay of both the spectrum was recorded. From the overlain spectra wavelengths 224 nm (λmax of MET) and 260 nm (λmax of AZD) were selected for analysis of both drugs using simultaneous method. (λ1-224 nm and λ2-260 nm).

The absorbance at λ1 and λ2 was measured and the concentration was calculated using following formula;

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

Where,

Cx and Cy are the concentrations of Metoprolol and Azelnidipine, respectively,

A1 and A2 are the absorbances of sample at λ1 and λ2, respectively,

ax1 and ax2 are the absorptivity of Metoprolol at λ1 and λ2, respectively,

ay1 and ay2 are the absorptivity of Azelnidipine at λ1 and λ2, respectively.

UV Method Validation

a. Linearity:

 i. 5 samples of varying concentrations ranging from 80% to 120% were made.

 ii. The concentrations are given below

 iii. The sample preparations are given as below;

 iv. X ml of BSSS-I and Y ml of MSSS-II was diluted to 10 ml.

X ml of MSSS-I	Y ml of ASSS-II	Diluted to	Conc. of MET (µg/ml)	Conc. of AZD (µg/ml)
0.08	0.08	10 ml	20	6.4
0.09	0.09	10 ml	22.5	7.2
0.10	0.10	10 ml	25	8
0.11	0.11	10 ml	27.5	8.8
0.12	0.12	10 ml	30	9.6

b. LOD/ LOQ:

Can be calculated by using AVONA Technique.

$$LOD = \frac{3.3 \times Std\ Error\ of\ Intercept}{Coefficient\ of\ X\ variable\ 1}$$

$$LOD = \frac{10 \times Std\ Error\ of\ Intercept}{Coefficient\ of\ X\ variable\ 1}$$

c. Repeatability :

A single sample was prepared as described and 6 injections were made from same sample; checked for RSD.

d. Accuracy:

i. Samples were made of 80%, 100% and 120% concentration as per Table 1.

ii. Samples were injected in triplicate to calculate % RSD.

iii. % recovery was also calculated.

% Conc	MET Conc. (µg/ml)	AZD Conc. (µg/ml)
80	12	36
100	15	45
120	18	54

d. Intra- & Inter-day Precision:

- The working standard and drug product samples were freshly prepared and analysed in morning and evening for Intra-day precision.
- The same working standard and drug product were used for analysis on 2nd day for inter-day precision.
- % RSD for Assay was calculated for the confirmation of precision.

RESULT :-

UV method

Selection of Wavelength

The Standard and Sample solution was scanned from 190 to 400 nm by using UV-VIS spectrophotometer against Diluent (0.1% Perchloric acid: Acetonitrile (50:50)) as blank and the maximum absorption of standard and sample solution were recorded.

Result:

a. The maximum absorption for Metoprolol was found to be 224 nm.

b. The maximum absorption for Azelnidipine was found to be 260 nm.
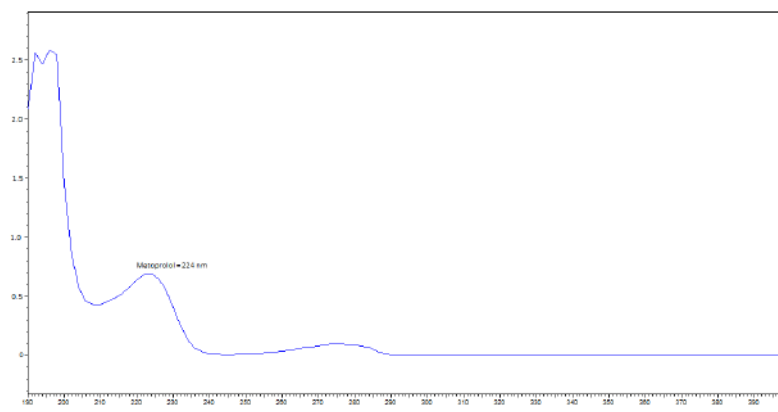
The UV scans for both the drugs is given below:

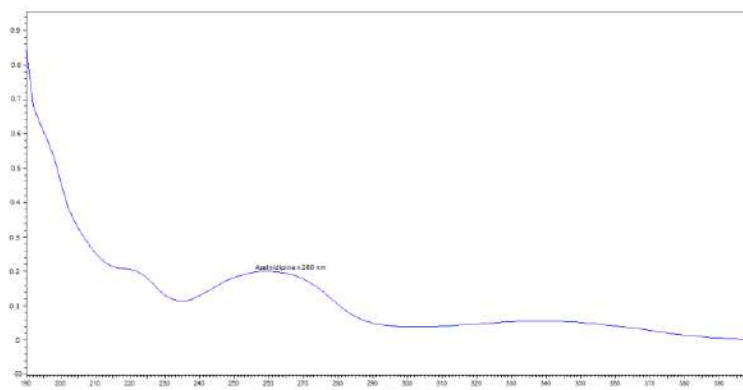

Figure 1: UV Scan of Metoprolol

Figure 2: UV Scan of Azelnidipine

UV Method Validation of Metoprolol and Azelnidipine

a. Specificity

It was confirmed with blank and working standard run that there was zero absorbance of blank at set lambda in UV Spectrophotometer.

b. Linearity

The peak response is directly proportional to the concentration of drug and was found to be linear in the range of 8-12µg/ml.

The linearity data for Metoprolol and Azelnidipine is give below:

Table 2: Linearity data for Metoprolol

% Level	Concentration (ug/ml)	Absorbance
80	20	0.588
90	22.5	0.659
100	25	0.734
110	27.5	0.809
120	30	0.891

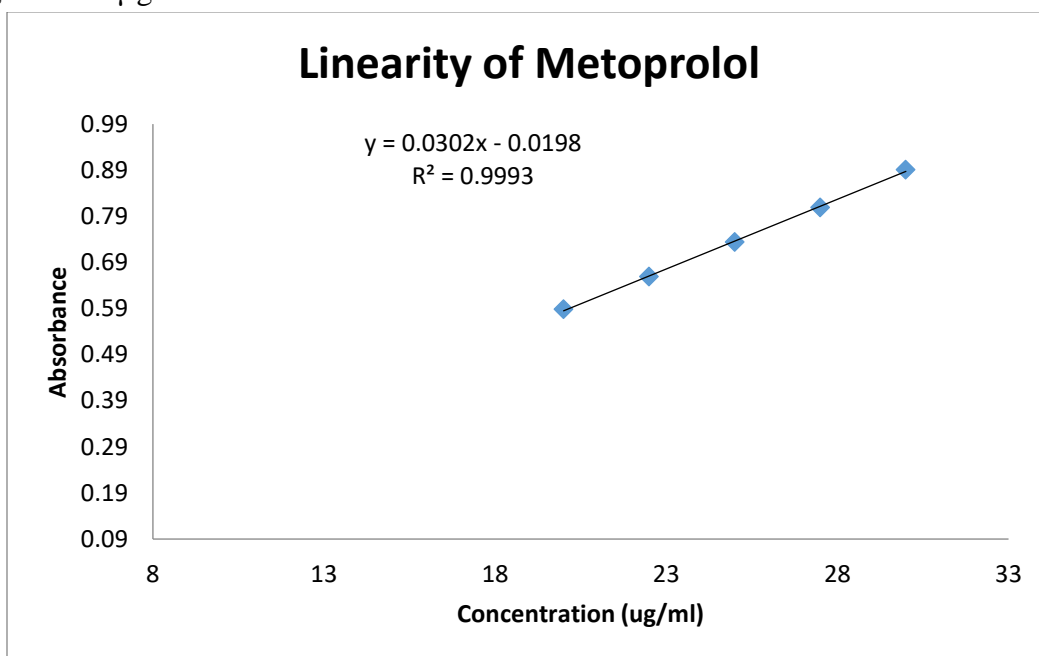**Figure 3: Linearity graph of Metoprolol**

Table 3: Linearity data for Azelnidipine

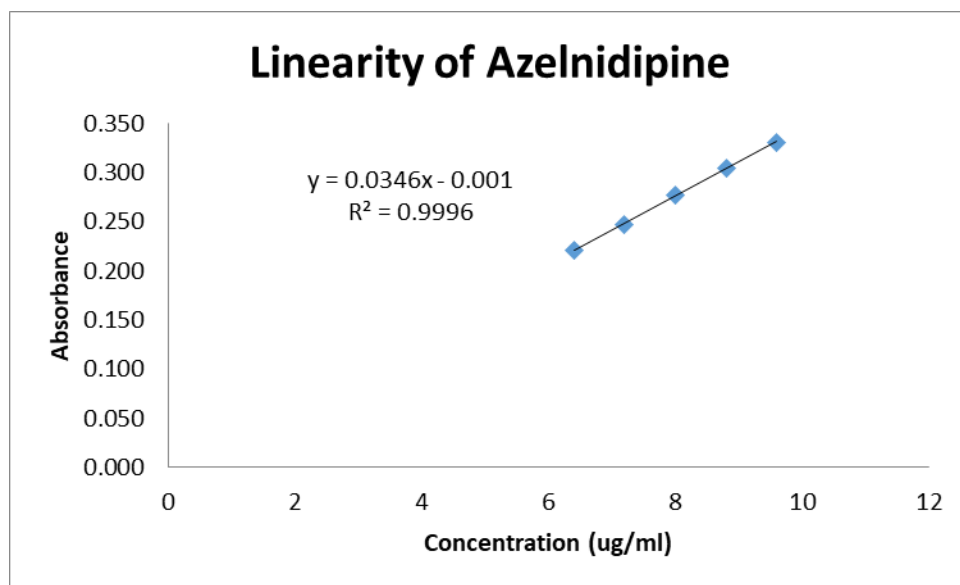| % Level	Concentration (ug/ml)	Absorbance
80	6.4	0.221
90	7.2	0.247
100	8	0.277
110	8.8	0.304
120	9.6	0.331

Figure 4: Linearity graph of Azelnidipine

From the above data it was found that the correlation coefficient was found to be 0.999 for both the drugs i.e. Metoprolol and Azelnidipine respectively, which was found to be within the acceptance criteria of 0.998.

C. LOD and LOQ

Based on the linearity data, LOD and LOQ was calculated and reported as below:

Table 4: LOD & LOQ of Metoprolol

Regression Statistics	
Multiple R	0.99967472
R Square	0.999349546
Adjusted R Square	0.999132728
Standard Error	0.003521363
Observations	5

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.0571536	0.0571536	4609.16129	7.04204E-06
Residual	3	3.72E-05	1.24E-05		
Total	4	0.0571908			

	Coefficients	Standard Error	t Stat	P-value
Intercept	-0.0198	0.011246333	-1.76057391	0.176533624
X Variable 1	0.03024	0.000445421	67.89080417	7.04204E-06

LOD	1.23	ug/ml
LOQ	3.72	ug/ml

Table 5: LOD & LOQ of Azelnidipine

Regression Statistics	
Multiple R	0.999798052
R Square	0.999596144
Adjusted R Square	0.999461525
Standard Error	0.00101653
Observations	5

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.0076729	0.0076729	7425.387097	3.44494E-06
Residual	3	3.1E-06	1.03333E-06		
Total	4	0.007676			

	Coefficients	Standard Error	t Stat	P-value
Intercept	-0.001	0.003246537	-0.308020552	0.778214513
X Variable 1	0.034625	0.000401819	86.17068583	3.44494E-06

LOD	0.31	ug/ml
LOQ	0.94	ug/ml

From the above data it was found that:

1. The LOD & LOQ for Metoprolol were found to be 1.23µg/ml and 3.72 µg/ml.
2. The LOD & LOQ for Azelnidipine were found to be 0.31 µg/ml and 0.94 µg/ml.

d. Repeatability

Repeatability was performed for both the APIs, the recorded absorbance is shown below:

Table 6: Repeatability of Metoprolol and Azelnidipine

Sample ID	MET ABS	AZD ABS
100% Rep 1	0.734	0.277
100% Rep 2	0.735	0.275
100% Rep 3	0.738	0.278
100% Rep 4	0.739	0.274
100% Rep 5	0.731	0.279
100% Rep 6	0.736	0.275
AVG	0.736	0.276
STDEV	0.003	0.00
%RSD	0.39	0.71

From the above data, it can be seen that the %RSD for 6 replicate injections of Metoprolol and Azelnidipine are 0.39% and 0.71% respectively. The percentage RSD (<2) values obtained showed

that the method developed was precise at repeatability precision level.

e. Accuracy

The accuracy was performed at 3 different levels i.e. 80%, 100% and 120%. The accuracy data for Metoprolol and Azelnidipine is given below:

Table 7: Accuracy of Metoprolol

% Level	Reps	Spiked Conc (ug/ml)	Abs	Amount Recovered (ug/ml)	% Recovery	AVG	STD EV	%RSD	% Level
80	Rep 1	20.00	0.588	19.97	99.86	Rep 1	99.81	0.60	0.60
	Rep 2	20.00	0.591	20.07	100.37	Rep 2			
	Rep 3	20.00	0.584	19.84	99.18	Rep 3			
100	Rep 1	25.00	0.734	24.93	99.73	Rep 1	99.95	0.28	0.28
	Rep 2	25.00	0.735	24.97	99.86	Rep 2			
	Rep 3	25.00	0.738	25.07	100.27	Rep 3			
120	Rep 1	30.00	0.891	30.26	100.88	Rep 1	100.85	0.62	0.62
	Rep 2	30.00	0.896	30.43	101.45	Rep 2			
	Rep 3	30.00	0.885	30.06	100.20	Rep 3			

- The %RSD of three replicates of Metoprolol for accuracy level 80%, 100% and 120% was found to be 0.60%, 0.28% and 0.62% respectively.

- The % recoveries for accuracy level 80%, 100% and 120% was found to be 99.81%, 99.95% and 100.85% respectively.

Table 8: Accuracy of Azelnidipine

% Level	Reps	Spiked Conc (ug/ml)	Abs	Amount Recovered (ug/ml)	% Recovery	AVG	STD EV	RSD	% Level
80	Rep 1	6.40	0.221	6.41	100.09	Rep 1	99.34	0.69	0.70
	Rep 2	6.40	0.219	6.35	99.18	Rep 2			
	Rep 3	6.40	0.218	6.32	98.73	Rep 3			
100	Rep 1	8.00	0.277	8.03	100.36	Rep 1	100.24	0.55	0.55
	Rep 2	8.00	0.275	7.97	99.64	Rep 2			
	Rep 3	8.00	0.278	8.06	100.72	Rep 3			
120	Rep 1	9.60	0.331	9.59	99.94	Rep 1	99.94	0.60	0.60
	Rep 2	9.60	0.329	9.54	99.34	Rep 2			
	Rep 3	9.60	0.333	9.65	100.54	Rep 3			

- The %RSD of three replicates of Azelnidipine for accuracy level 80%, 100% and 120% was found to be 0.70%, 0.55% and 0.60% respectively.

- The % recoveries for accuracy level 80%, 100% and 120% was found to be 99.34%, 100.24 % and 99.94% respectively.

f. Intra & Inter day Precision

The Standard solution of Metoprolol and Azelnidipine were examine for Intra and Inter day Precision, the data is shown below:

Table 9: Intra & Inter day Precision of Metoprolol and Azelnidipine

Condition	Sample ID	Interval	Metoprolol		Azelnidipine	
			Conc (ug/ml)	% Assay	Conc (ug/ml)	% Assay
Intraday	WS	Mrng	25.00	-	8.00	-
	DP	Mrng	25.03	100.12	7.98	99.75
	WS	Evng	25.00	-	8.00	-
Interday	DP	Evng	24.94	99.76	7.94	99.25
	WS	Day 2	25.00	-	8.00	-
	DP	Day 2	24.93	99.72	7.96	99.50
% RSD				0.22	% RSD	0.25

- The % Assay for Metoprolol for Morning, Evening and Day 2 were found to be 100.20%, 99.76% and 99.72%, respectively.
- The % Assay for Azelnidipine for Morning, Evening and Day 2 were found to be 99.75%, 99.25% and 99.50%, respectively.
- The %RSD for intra and Inter day Precision of Metoprolol and Azelnidipine were found to be 0.22% and 0.25%, respectively.

- Hence, the working standard Metoprolol and Azelnidipine is stable for 2 days as no significant variation was observed.

G. Assay

The data of the Assay of Metoprolol and Azelnidipine is given below:

Table 10: Assay of Metoprolol and Azelnidipine

Sample	Metoprolol		Azelnidipine	
	Conc (ug/ml)	% Assay	Conc (ug/ml)	% Assay
DP-1	24.98	99.92	8.03	100.38
DP-2	24.93	99.72	8.04	100.50
DP-3	25.04	100.16	7.95	99.38
DP-4	25.06	100.24	7.98	99.75
DP-5	24.91	99.64	7.94	99.25
AVG		99.94	AVG	99.85
STDEV		0.26	STDEV	0.57
%RSD		0.26	%RSD	0.57

- The average assay for Metoprolol and Azelnidipine were found to be 99.94% and 99.85% respectively.
- The %RSD for assay of Metoprolol was found to b 0.26%.
- The %RSD for assay of Metoprolol was found to b 0.57%.

CONCLUSION

The UV method developed for the estimation of Metoprolol and Azelnidipine was validated as per the ICH guidelines. Validation data demonstrates that, these methods are accurate, precise, simple, and economic and can be used in the routine analysis of Metoprolol and Azelnidipine in various formulations.

REFERENCE

1. Harris DC. Quantitative Chemical Analysis. 7th ed, 3rd printing. W. H. Freeman; 2007.
2. Diffey BL. Sources and measurement of ultraviolet radiation. Methods. 2002;28(1):413.
3. Namioka T. Diffraction Gratings. In: Vacuum Ultraviolet Spectroscopy. Vol 1.

Experimental Methods in Physical Sciences. Elsevier; 2000:347-377.

4. Mortimer Abramowitz and Michael W. Davidson. Photomultiplier Tubes. Molecular Expressions. Accessed April 25, 2021. https://micro.magnet.fsu.edu/primer/digitali maging/concepts/photomultipliers.html

5. Picollo M, Aceto M, Vitorino T. UV-Vis spectroscopy. Phys Sci Rev. 2019;4(4). doi:10.1515/psr-2018-0008

6. What is a Photodiode? Working, Characteristics, Applications. Published online October 30, 2018. Accessed April 29, 2021. https://www.electronicshub.org/photodiode-working-characteristics-applications/

7. Amelio G. Charge-Coupled Devices. Scientific American. 1974;230(2):22-31. http://www.jstor.org/stable/24950003

8. Hackteria. DIY NanoDrop. Accessed June 15, 2021. https://hackteria.org/wiki/File:NanoDropCon ceptSpectrometer2.png

9. Sharpe MR. Stray light in UV-VIS spectrophotometers. Anal Chem. 1984;56(2):339A-356A.

10. Liu P-F, Avramova LV, Park C. Revisiting absorbance at 230nm as a protein unfolding probe. Anal Biochem. 2009;389(2):165-170.

11. Kalb V., Bernlohr R. A New Spectrophotometric Assay for Protein in Cell Extracts. Anal Biochem. 1977;82:362-371.

12. Bosch Ojeda C, Sanchez Rojas F. Recent applications in derivative ultraviolet/visible absorption spectrophotometry: 2009–2011. Microchem J. 2013;106:1-16.

13. Domingo C, Saurina J. An overview of the analytical characterization of nanostructured drug delivery systems: Towards green and sustainable pharmaceuticals: A review. Anal Chim Acta. 2012;744:8-22.

14. Kristo E, Hazizaj A, Corredig M. Structural Changes Imposed on Whey Proteins by UV Irradiation in a Continuous UV Light Reactor. J Agric Food Chem. 2012;60(24):6204-6209

15. Lange R, Balny C. UV-visible derivative spectroscopy under high pressure. Biochim Biophys Acta BBA - Protein Struct Mol Enzymol. 2002;1595(1-2):80-93.