Original Article

Development And Validation Of Stability Indicating Rp-Hplc Method For Quantitative Estimation Of Metoprolol Succinate And Azelnidipine From Synthetic Mixture

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Abstract

A new simple, precise, accurate and selective stability indicating RP-HPLC method has been developed and validated for estimation of Metoprolol succinate and Azelnidipine in synthetic mixture. The method was carried out on Hypersil ODS C_{18} 5µ column (250 x 4.6 mm) with a mobile phase consisting of Acetonitrile: 0.025 M KH_2PO_4 Buffer (70:30 v/v, pH adjusted to 3 with 10% Ortho phosphoric acid and flow rate of 1 mL/min. Detection was carried out at 228 nm. The retention time for Metoprolol succinate and Azelnidipine was found to be 3.281 min and 10.799 min, respectively. The Metoprolol succinate and Azelnidipine followed linearity in the concentration range of 25-125 µg/mL (r^2= 0.9995) and 8-40 µg/mL (r^2=0.9997) . The developed method was validated for linearity and range, accuracy, precision, and assay. Metoprolol Succinate and Azelnidipine was subjected to acid and alkali hydrolysis, oxidation and thermal degradation. This indicates that the drug is susceptible to acid, base, oxidation and thermal conditions. The degraded product was well resolved from the pure drug with significantly different Retention Time. The proposed method can be used for routine analysis of Metoprolol Succinate and Azelnidipine in synthetic mixture.

Keywords: Metoprolol Succinate, Azelnidpine, RP-HPLC method, Validation, Force degradation

INTRODUCTION

Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors. This inhibition decreases cardiac output by producing negative chronotropic and inotropic effects without presenting activity towards membrane stabilization nor intrinsic sympathomimetics.[1-7] Azelnidipine inhibits Ca^{2+} influx through the voltage dependent channels of smooth muscles in vascular walls. It results in relaxation ofvascular smooth muscle walls and decreased blood pressure.[8-10] The combination of these two drugs Metoprolol Succinate and Azelnidipine is indicated for stage 2 hypertension. A clinical trial has been conducted with the dose of Metoprolol succinate 50mg in combination with Azelnidipine 16mg. Several analytical methods are available which can determine MET and AZE individually or in combination with another drug. From detailed review of literature, it was found that no analytical method is available for determination of MET and AZE and its degradants from simulated mixture or formulation [11-26]. So for the same reason stability indicating RP – HPLC method was selected.

MATERIAL AND METHODS

MET (99.98% pure) and AZE (99.96% pure) were obtained as gift sample for research purpose from, Cadila Healthcare Ltd., Sanand. Acetonitrile (HPLC grade), Orthophosphoric acid (LR grade) was purchase from S.D. fines.

Preparation of stock solution

For the method development purpose, 50 mg of MET was weighed and diluted to 10 mL with methanol (5000 µg/mL) and was further diluted with mobile phase to give final concentration of 50 µg/mL. In similar way 16 mg of AZE was weighed and diluted to 10 ml methanol (1600 µg/mL) and was further diluted with mobile phase to give final concentration of 16 µg/mL.

Selection of analytical wavelength

The working standards of MET (50 µg/mL) and AZE (16 µg/mL) were prepared in 10 mL volumetric flask using methanol as a solvent. They were scanned in the UV range of 200 – 400 nm.

PREPARATION OF SOLUTIONS FOR FORCED DEGRADATION STUDIES

Acid induced hydrolysis

Accurately weighed amount corresponding to 50 mg of MET and 16 mg of AZE were transferred to 10 ml volumetric flask and add 5 mL of 1 N HCl. Same solution was heated under reflux condition at 60°C for 1 hour on a hot plate. After the heating cool down the solution and were neutralized with 2 N NaOH and volume was raised to mark with diluent if necessary. 0.1 mL of previous solution was further diluted to 10 mL with diluent. The resulting solution have concentration of 50 µg/mL of MET and 16 µg/mL of AZE (Treated sample). In similar way 0 hour sample (Only difference was heating condition was not provided) and blank (Only difference is there is no addition of API) were prepared. % Degradation of both components was calculated by comparing area of treated sample and control.

Base induced hydrolysis

Same amount of API like in former case were transferred to 10 mL volumetric flask and volume of same was raised to the mark with 5 mL 1 N NaOH Same solution was heated under reflux condition at 60°C for 1 hour on a hot plate. After the heating cool down the solution and were neutralized with 2 N HCl and volume was raised to mark with diluent if necessary. 0.1 ml of previous solution was further diluted to 10 mL with diluent. The resulting solution have concentration of 50 µg/mL of MET and 16 µg/mL of AZE (Treated sample). In similar way 0 hour sample and blank sample were prepared. % Degradation of both components was calculated by comparing area of treated sample and control.

Hydrogen peroxide induced stress (Oxidative)

Same amount of API like in former case were transferred to 10 mL volumetric flask and volume of same was raised to the mark with 5 mL 3% hydrogen peroxide. Same solution was heated under reflux condition at 60°C for 1 hour on a hot plate. After the heating cool down the solution and volume was raised to mark with diluent. 0.1 ml of previous solution was further diluted to 10 mL with diluent. The resulting solution have concentration of 50 µg/mL of MET and 16 µg/mL of AZE (Treated sample). In similar way 0 hour sample and blank sample were prepared. % Degradation of both components was calculated by comparing area of treated sample and control.

Thermal stress

Exact quantity of MET and AZE like in previous cases were transferred to petri dish and exposed to 70 C° for 3 hours in hot air oven and residues were reconstituted with help of acetonitrile and transferred into 10 mL volumetric flask and volume of flask was raised with the mark with same solvent. 0.1 mL of resulting solution was further diluted to 10 ml with diluent. Above solution was chromatographed and % degradation was computed by comparing against standard concentration of MAT and AZE.

PREPARATION OF SOLUTIONS FOR ANALYTICAL METHOD VALIDATION

Linearity and range

To study linearity and range, accurately weighed quantities of 50 mg MET and 16 mg AZE was diluted to 100 mL with diluent to produce stock solution containing 500 µg/mL MET and 160 µg/mL of AZE. Various aliquotes were transferred to 10 mL volumetric flask and volume of each raised to 10 mL to give mixtures having concentration of 25-125 µg/mL and 8-40 µg/mL of MET and AZE, respectively. Each concentration was injected at 20 µl injector volume and response obtained was plotted against concentration to observe linear regression coefficient.

Intermediate precision (Repeatability)

For adjudging repeatability of method, solution of linearity studies were analyzed 5 times and each level is observed for relative standard deviation (RSD). Method Precision This parameter was studies by injecting individual concentration that represents overall range are studies on same day and between days and for the same Mixture of MET and AZE that represents overall range (25+8, 75+24 and 125+40 µg/mL) were analyzed on same day at different time interval for Intraday precision and different day for Interday precision. Each concentration was analyzed for three times and was monitored for RSD at each level.

Accuracy

As it refers to the % recovery of analyte in presence of excipients, it was practiced by spiking of placebo with standard at 50, 100 and 150% of target concentration. Each concentration was injected for three times and % recovery was calculated on the basis of served area at each spiking level (By utilization of linear regression analysis). Composition of placebo: HPMC (4 mg) - Film forming agent, MCC (190 mg) - Directly compressible material, Magnesium stearate (4 mg) - Gliding agent and Talc (2 mg) - Lubricating agent. (Table 1)

Assay

For the said purpose, 50 mg of MET and 16 mg of AZE were weighed accurately and mixed with commonly used excipient (same used in accuracy study). All the contents were diluted to 10 mL with acetonitrile, sonicated for 5 minutes and filtered from 0.45 micron membrane whatman filter paper. 0.1 mL of previously filtered solution was further diluted

to 10 mL with diluent (mobile phase) to give final concentration of 50 µg/mL of MET and 16 µg/mL of AZE. Above solution was chromatographed in triplicates by employing optimized chromatographic conditions.[27-30]

RESULT AND DISCUSSION

Selection of analytical wavelength

Working standard of Metoprolol and azelnidipine were scanned in UV range of 200 – 400 nm and overlapped Two iso-absorptive points were observed that is 219 nm and 228 nm (Figure 1) 219 nm is nearby wavelength of methanol cut off. Therefore, 228 nm was selected as analytical wavelength for further trials. As well as both the compounds gives good intensity peak at 228 nm.

OPTIMIZED CHROMATOGRAPHIC CONDITION

When method was operated using optimized chromatographic condition a well resolved peak of MET and AZE was observed at 3.281 and 10.799 minutes respectively (Figure 2). All the system suitability parameters were within the guidelines.

FORCED DEGRADATION STUDIES

Optimized method was found to be stability indicating as it is able to separate all the degradation products in the presence of active ingredient. (Figure 3 – 6) No degradation product found to interfere with estimation of MET and AZE in stressed samples. Even the stress given found to be optimum as % degradation observed was predictive in nature (below 15%). (Table 3)

ANALYTICAL METHOD VALIDATION

Linearity and Range

As per ICH guidelines, the value of r^2 should be greater than 0.995 and observed r^2 for given concentration range for MET and AZE is 0.9995 and 0.9997 respectively. Hence, we can say that developed method is linear over the range of 25 – 125 µg/mL and 8 – 40 µg/mL for MET and AZE respectively show in Figure 7 and 8. Linearity data for both drugs is shown in Table 4 and 5.

Repeatability

When all mixtures were analyzed at all concentration, calculated relative standard deviation at each level was found to be less than 2 so that method was found to be repeatable over the range of 25 – 125 µg/mL and 8 – 40 µg/mL for MET and AZE respectively. Repeatability data are shown in Table 6 and 7 for MET and AZE respectively.

Method precision

For determining inter day and intraday precision, %RSD was monitored at selected concentration level which was found to be less than 2 so method was found to be precise for estimation of MET and AZE. Data for intermediate precision are given in Table 8 and 9 for MET and AZE respectively.

Accuracy study

Accuracy of the analytical method has been performed by spiking of placebo with the standard. Placebo for the study was selected on the basis of reported formulation. And spiking of the placebo was performed at 50,100 and 150 % of the target concentration. (Table 10, 11).

Assay

When prepared synthetic mixture was analyzed by developed and validated method, % assay was found to be 99.435 for ASP and for 99.416 PAN (Table 12)

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Author Contribution

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Table 1: Preparation of solution for accuracy studies

Concertation of stock solution	**5000 µg/ml of MET and 1600 µg/ml of AZE**			
Volume taken from SS	-	50 µL	100 µL	150 µL
Quantity of Placebo added	200 mg	200 mg	200 mg	200 mg
Volume made up with	10 ml	10 ml	10 ml	10 ml
Diluent	Mobile phase	Mobile phase	Mobile phase	Mobile phase
Final Concentration	-	25+8 µg/ml	50+16 µg/ml	75+24 µg/ml
Identification	Unspiked	50 % Spiked	100 % Spiked	150 % Spiked

Table 2: System suitability parameter of MET and AZE

Parameter	**MET**	**AZE**
Retention time (Rt)	3.279 ± 0.007	10. 791 ± 0.011
Tailing factor (T)	1.12 ± 0.01	1.42 ± 0.015
Number of theoretical plates (N)	3677 ± 30.80	11241 ± 100.71
Resolution (Rs)	10.25 ± 0.056	

Table 3: Evaluation Table of Forced Degradation Studies

Stress Condition	**Area**	**MET**	**AZE**	**% Degradation (MET)**	**% Degradation (AZE)**
Acid Hydrolysis	Standard Area	846742	1357131	13.09 %	15.27 %
	Observed Area	735894	1149874		
Base Hydrolysis	Standard Area	846742	1357131	14.32 %	17.28 %
	Observed Area	725478	1122543		
Oxidative Stress	Standard Area	846742	1357131	12.06 %	13.67 %
	Observed Area	744612	1171562		
Thermal Degradation	Standard Area	846742	1357131	11.5 %	12.36 %
	Observed Area	749314	1189325		

Table 4: Linearity data of MET

Sr. No.	**Concentration**	**Mean area ± SD**	**RSD**
1	25	428564.8 ± 5191.47	1.21
2	50	863654 ± 10303.55	1.19
3	75	1290893 ± 14741.85	1.14
4	100	1662043 ± 21681.88	1.30
5	125	2122549 ± 23015.38	1.08

Table 5: Linearity data of AZE

Sr. No.	**Concentration**	**Mean area ± SD**	**RSD**
1	8	664673.6 ± 8625.91	1.30
2	16	1342729 ± 14949.95	1.11
3	24	1952711 ± 21178.56	1.08
4	32	2583978 ± 43274.05	1.67
5	40	3217435 ± 29275.29	1.91

Table 6: Repeatability data of MET

Concentration	**25**	**50**	**75**	**100**	**125**
Area 1	424952	846742	1287234	1646124	2124125
Area 2	434348	864622	1267323	1682414	2099643
Area 3	424786	865723	1295753	1688691	2098681
Area 4	434151	866281	1298432	1643472	2144581
Area 5	424587	874902	1305722	1649512	2145713
Mean	428564.8	863654	1290893	1662043	2122549
SD	5191.47	10303.55	14741.85	21681.88	23015.38
RSD	**1.21**	**1.19**	**1.14**	**1.30**	**1.08**

Table 7: Repeatability data of AZE

Concentration	8	16	24	32	40
Area 1	674242	1357131	1988351	2653212	3246123
Area 2	667571	1345713	1945533	2597571	3199874
Area 3	669312	1322556	1952545	2546233	3217153
Area 4	659987	1355673	1932563	2567417	3245462
Area 5	652256	1332571	1944561	2555456	3178561
Mean	664673.6	1342729	1952711	2583978	3217435
SD	8625.91	14949.95	21178.56	43274.05	29275.29
RSD	**1.30**	**1.11**	**1.08**	**1.67**	**0.91**

Table 8: Intraday and Interday precision data of MET

Concentration (µg/ml)	IntradayMean	± SD(n=3)	RSD	Inter-Day Mean	± SD(n=3)	RSD
25	431990	6464.14	1.50	431980	6364.14	1.47
75	1292646.66	17757.27	1.37	1292346	17757.27	1.35
125	2228676	28921.40	1.30	2218675	27901.40	1.80

Table 9: Intraday and Interday precision data of AZE

Concentration (µg/ml)	IntradayMean	± SD (n=3)	RSD	Inter-Day Mean	± SD (n=3)	RSD
8	72753.666	728.88	1.00	656079.66	10554.78	1.61
24	279564.333	2945.02	1.05	1916384	19789.12	1.03
40	1155217.667	9342.30	0.81	3219143.667	32037.76	1.01

Table 10: Accuracy data of MET by HPLC method

Level of spiking	Quantity of placebo (mg)	Amount of drug added (µg/mL)	Amount ofdrug recovered (µg/mL)	% Recovery	% Mean Recovery ±SD (n=3)
50%	200	25	24.75	99	100.56 ±1.42
		25	25.22	100.88	
		25	25.45	101.8	
100%	200	50	50.44	100.88	100.347 ±0.57
		50	49.87	99.74	
		50	50.21	100.42	
150%	200	75	75.22	100.29	99.86 ±0.634
		75	74.35	99.13	
		75	75.12	100.16	

Table 11: Accuracy data of AZE by HPLC method

Level of spiking	Quantity of placebo (mg)	Amount of drug added (µg/mL)	Amount ofdrug recovered (µg/mL)	% Recovery	% Mean Recovery ±SD (n=3)
50%	200	8	7.77	97.125	100.75 ±3.314
		8	8.29	103.625	
		8	8.12	101.5	
100%	200	16	16.08	100.5	100.85 ±1.74
		16	15.89	99.3125	
		16	16.44	102.75	
150%	200	24	24.12	100.5	100.54 ±0.48
		24	24.25	101.04	
		24	24.02	100.08	

Table 12: Assay of MET and AZE by HPLC method

Drug	Amount taken (µg/ml)	Amount found (µg/ml) (n=5)	% Assay (n=5)
MET	50	49.82± 0.69	99.64 ± 1.38
AZE	16	16.22 ± 0.06	101.37 ± 0.40

List of Figures

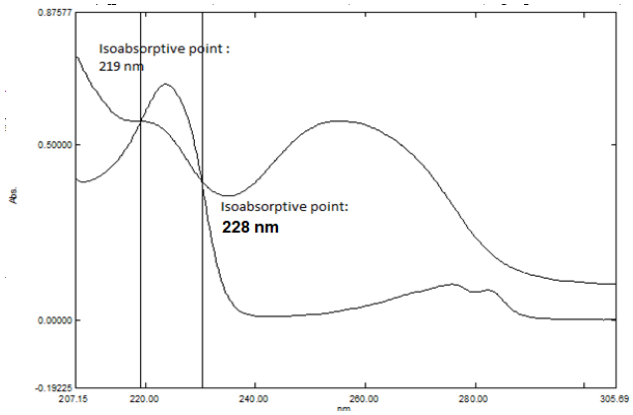

Figure 1: Overlain UV Spectra of AZE (10 µg/mL) and MET (50 µg/mL)

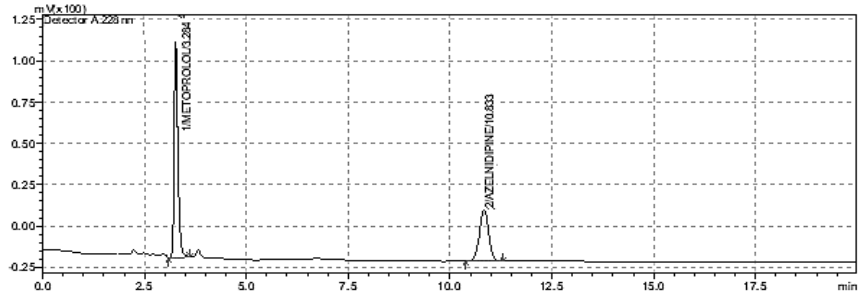

Figure 2: Chromatogram of mixture of MET and AZE using optimized chromatographic

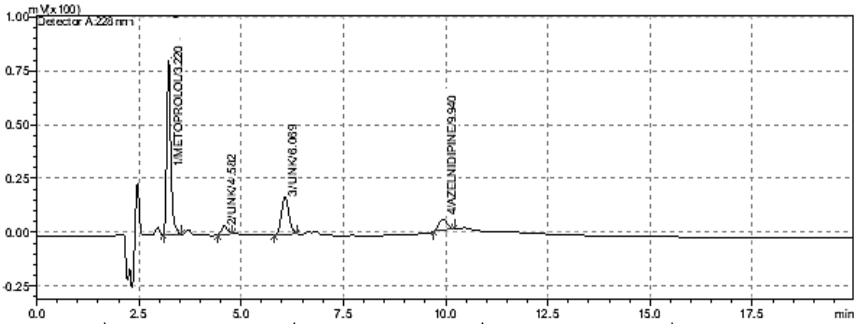

Figure 3: Chromatogram of treated sample (Acid hydrolysis)

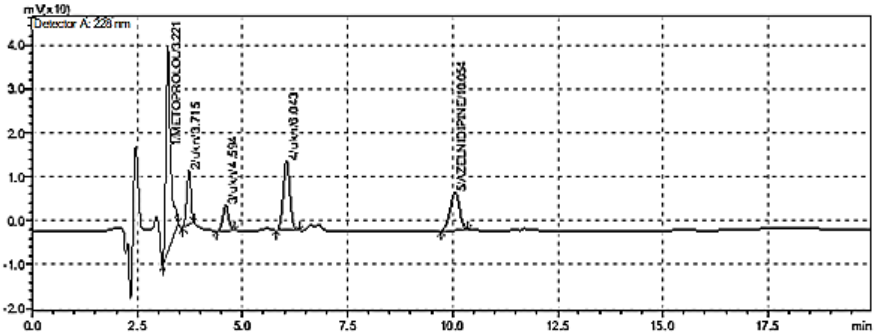

Figure 4: Chromatogram of treated sample (Base hydrolysis)

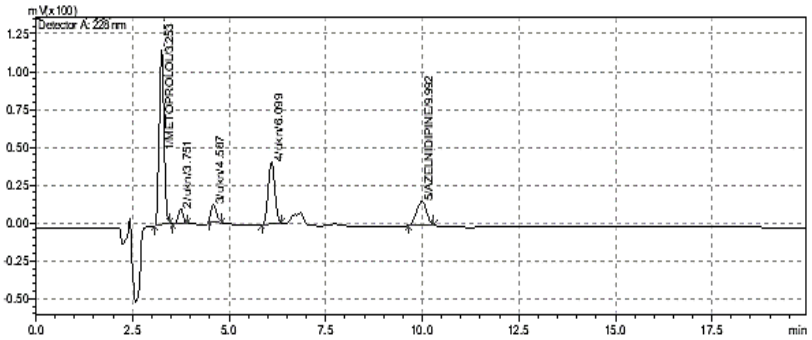

Figure 5: Chromatogram of treated sample (Oxidative stress)

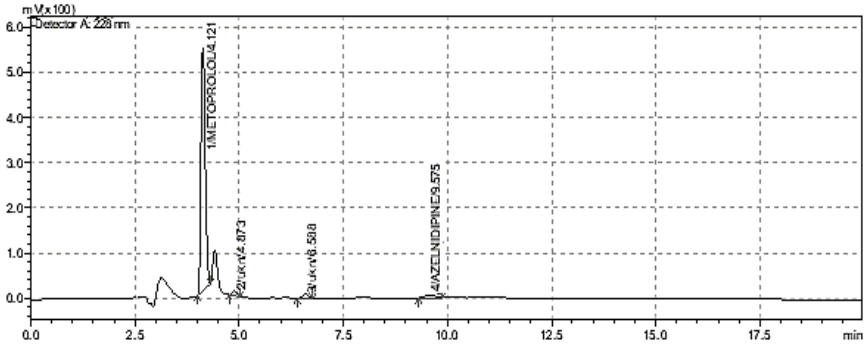

Figure 6: Chromatogram of treated sample (Thermal stress)

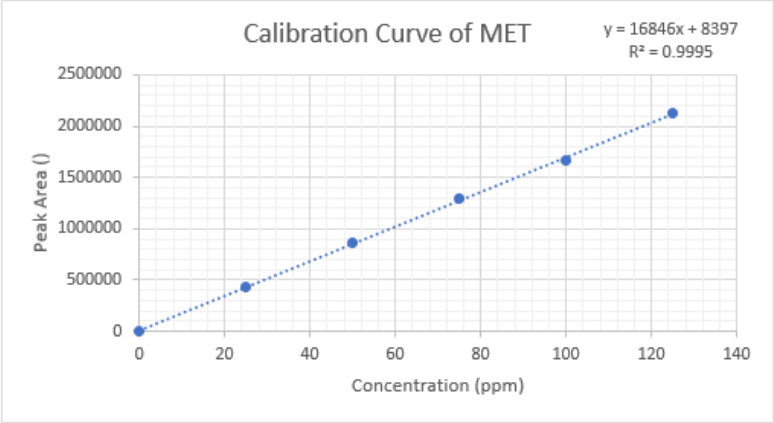

Figure 7: Regression analysis of MET (25 – 125 µg/mL)

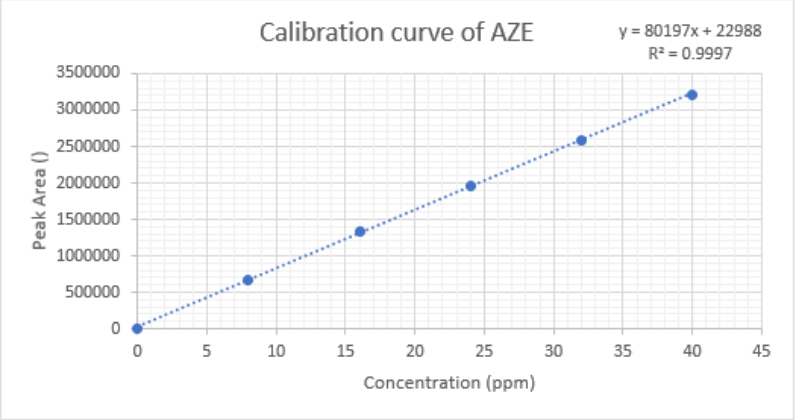

Figure 8: Regression analysis of AZE (8 – 40 µg/mL)

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