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Research Article

Method Development, Validation and Stability Indicating Studies for Simultaneous Estimation of Anti-Hypertensive Drugs from Pharmaceutical Formulation by RP-HPLC

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ABSTRACT

Objective: Method development, validation & stability indicating studies for simultaneous estimation of Anti-Hypertensive drugs, Benidipine (BEN) and Metoprolol (MET) from pharmaceutical formulation by RP-HPLC.

Methods: For present work, reverse phase chromatography was selected as its suggested use for ionic and moderate to non-polar compounds. Reverse phase chromatography is simple, suitable, better regarding efficiency, stability, and reproducibility. C18 packed column, a 100 X 2.1mm. ID column of 5.0 µm particle packing, was selected for separation of BEN and MET. Different solvent systems were tried and optimized in combinations as mobile phase. BEN (4 µg/ml) and MET (50 µg/ml) in 15mM ammonium formate-Methanol (15:85 v/v) was developed as it was showing good peak shapes and a significant amount of resolution. The mobile phase was flowed at 1.2 ml/min with detection of BEN analytes at 236 nm and MET analytes at 225 nm respectively.

Result: Method development was done. Specificity, linearity, accuracy, precision, robustness, limit of detection and limit of quantitation were used to accomplish validation. The method was found linear from 32.5 – 500 µg.ml⁻¹ for both BEN and MET individually. The percentage recovery of BEN when placed for period of 12 hours was found to 100% in 0.1N/M NaOH at 60°C and Thermal (60°C); 12 % degradation in 0.1N/M HCl at 60°C; Oxidation (3-6% H₂O₂) at room temperature whereas for MET was 100 % in 0.1N/M NaOH, 0.1N/M HCl at 60°C, at thermal (60°C) as well as oxidation by 3-6% H₂O₂ at room temperature.

Conclusion: Developed analytical method for the simultaneous estimation of Benidipine (BED) and Metoprolol (MET) in both bulk and tablet formulation has obliged the ICH guidelines including, tailing factor (*T*), separation factors (α), theoretical plates (*N*), capacity factor (*k'*), resolution (*R*) and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid ascertained few degradation products for Benidipine whereas the Metoprolol was unaffected with forced degradation studies.

Keywords: Benidipine, Metoprolol, Reverse-Phase High Performance Liquid Chromatography, Stability indicating method.

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1. INTRODUCTION

New analytical technologies that are continuously being developed and also been used when it is appropriate to develop stability indicating method. The unknown impurity, which is observed during the analysis, pharmaceutical development, stress studies and formal stability studies of

the drug substances and drug product, can be separated and analyzed by using various chromatographic techniques like reversed phase high performance liquid chromatography (RP-HPLC)^{1,2}.

Importantly, few publications reported the simultaneous analysis of both Benidipine and Metoprolol on C18 column³

and has mentioned the details of capacity factor and resolution which specifically have great importance in system suitability as per ICH guidelines. As reported in few articles the Metoprolol was eluted with void volume/solvent front (t_0) which is strictly not acceptable by ICH guidelines. In addition, the sensitivity of both Metoprolol and Benidipine were found negligible in UV detection⁴. Considering it, attempt has been made to develop new, accurate, precise and robust reverse phase high performance liquid chromatographic (RP-HPLC) method has been successfully developed for the simultaneous estimation of both antihypertensive drugs Benidipine^{5,6} (3R)-1-Benzyl-3-piperidinyl methyl (4R)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (BEN, Fig. 1) and Metoprolol^{7,8,9} 1-(Isopropylamino)-3-(4-(2-methoxyethyl) phenoxy) propan-2-ol (MET, Fig. 2) in both standard and tablet formulation along with stability indicating studies or

force degradation studies in 0.1 N HCl, 0.1N NaOH, 3% H₂O₂, and thermal degradation at 50°C temperature.

A stability indicating method¹⁰⁻¹⁵ (SIM) is an analytical procedure used to quantitate the decrease in the amount of the active pharmaceutical ingredient (API) in drug product due to degradation. SIM measures the changes in active ingredients concentration without interference from other degradation products, impurities and excipients. Stress testing is carried out to demonstrate specificity of the developed method to measure the changes in concentration of drug substance when little information is available about potential degradation product. The addition of this analytical methods in the current practice would help the pharmaceutical industries in large to preserve the excellence of their products containing these active ingredients and also the enforcement agencies in general to monitor the quality of the marketed products.

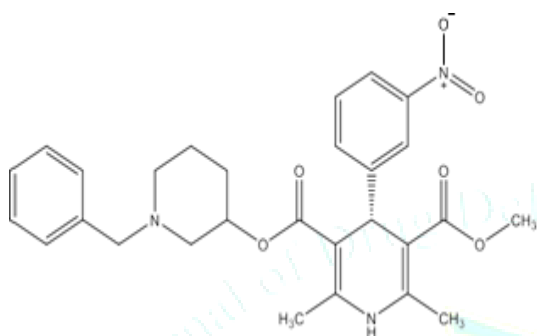


Figure 1: Molecular structure of Benidipine

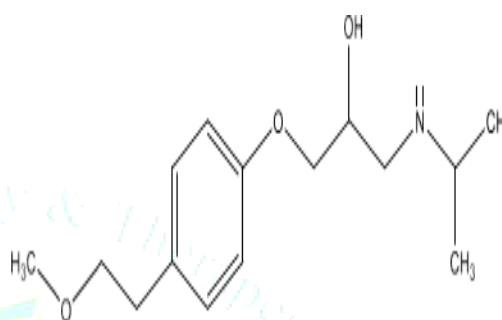


Figure 2: Molecular structure of Metoprolol

2. MATERIALS AND METHODS

Reagents and chemicals: Standard of Metoprolol and Benidipine were obtained from Intas Pharmaceuticals Pvt. Ltd., Ahmadabad. Benitowa@Beta (Akumentis Healthcare Ltd) tablets were purchased from medical store. BEN 4 mg and MET 25mg were used. All chemicals and reagents used were a HPLC grade and purchased from Merck specialities Pvt., Ltd., Mumbai.

Benidipine (BEN) standard stock solution (40 µg/ml)

A sample of 40 mg of BEN was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with methanol-water (2:1 v/v). Take 10 ml from this solution, and transfer to 100 ml volumetric flask and volume was made up with methanol-water (2:1 v/v).

Metoprolol (MET) standard stock solution (500 µg/ml)

A sample of 50 mg of MET was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with methanol-water (2:1 v/v).

Preparation of standard solution of binary mixtures of BEN (4 µg/ml) and MET (50 µg/ml)

Take 1 ml from the BEN stock solution and 1ml from MET stock solution and transferred to 10 ml volumetric flask and volume made up to the mark by mobile phase which was used in trials.

Preparation of Sample Stock Solution (BEN 40 µg/mL, MET 500 µg/ml)

Exactly 10 tablets of Benitowa@Beta, were separately weighed, powdered and mixed in a mortar. An accurately weighed amount of the finely powdered Benitowa@ Beta

4mg/50mg; Akumentis Healthcare Ltd tablets; equivalent to 4 mg of BEN and 50 mg of MET were separately made up to 100 mL with methanol and sonicated until they dissolved and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Method Validation¹⁶⁻²⁴

Linearity/Calibration studies

Accurately measured aliquots of stock solutions equivalent to 32.15-500 µg, of BEN and MET, respectively were transferred separately into a series of 10 mL volumetric flasks. The final volume was adjusted with same mobile phase, and then 20 µL were injected into HPLC. A calibration curve (linearity graph) was plotted by calculating peak area against concentration.

Precision of the proposed method

Three similar concentrations of the mixture of BEN and MET (500, 250, 125 µg.L⁻¹) were analyzed three times, within the same day (intraday precision), using the procedure mentioned under (5.7.1). Also, the mentioned concentrations were analyzed on three successive days using the same procedure to determine the intermediate precision.

Robustness

Robustness was attempted by deliberately changing the chromatographic conditions to evaluate the difference in resolution, capacity factor, peak height and peak width (tailing factor). The flow rate of the mobile phase was changed by ±2 decimal; like 1.2 mL.min⁻¹ was changed to 1.4 mL.min⁻¹ and 1 mL.min⁻¹ to evaluate the effect of the

flow rate; similarly the variation of organic modifier as Acetonitrile/methanol was changed by $\pm 2\%$ to 71% and 73% to monitor the peak area and retention time. Finally, the effect of wavelength was monitored by making deliberate variation 223 to 225 nm and the differences in system suitability parameters such as peak tailing, capacity factor, resolution and theoretical plates were evaluated.

Forced degradation studies²⁵

Acid degradation

Acid decomposition studies were performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. A volume of 2 ml of 0.1 N/M HCl solutions was added and mixed well and put for 12 hours at 60°C. After time period, the volume was adjusted with diluent to get 4 $\mu\text{g/ml}$ for BEN and 50 $\mu\text{g/ml}$ for MET.

Base degradation

Basic decomposition studies were performed by transferring 1ml of stock solution in to 10ml of volumetric flask. A volume of 2 ml of 0.1 N/M NaOH solutions was added and mixed well and put for 12 hours at 60°C. After time period, the volume was adjusted with diluents to get 4 $\mu\text{g/ml}$ for BEN and 50 $\mu\text{g/ml}$ for MET.

Oxidative degradation

Oxidation decomposition studies were performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. A volume of 2 ml of 3 - 6 % H_2O_2 solutions were added and mixed well and put for 12 hours at room temperature. After time period, the volume was adjusted with diluents to get 4 $\mu\text{g/ml}$ for BEN and 50 $\mu\text{g/ml}$ for MET.

Thermal degradation

Thermal degradation studies were performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 60°C for 12 hours. Then, the volume was adjusted with diluents to get 4 $\mu\text{g/ml}$ for BEN and 50 $\mu\text{g/ml}$ for MET.

3. RESULTS AND DISCUSSION

Selection of wavelength

Standard solution of BEN (4 $\mu\text{g/ml}$) and standard solution of MET (50 $\mu\text{g/ml}$) were scanned between 200nm and 400nm using UV-visible spectrophotometer Wavelength was selected from the overlay spectra of above solutions. UV detection was specifically carried out at 225nm for both selected BEN and MET as both compounds exhibit optimum absorption and showed good response at 225 nm. The flow rate was adjusted to 1.2 $\text{mL}\cdot\text{min}^{-1}$ to achieve better resolution, and peak symmetry.

Chromatographic Parameters²⁶

Various chromatographic parameters are as follows,

1. Analytes: Benidipine (250ppm) + Metoprolol (500ppm)
2. Column: UltraSil-MCX; 5 μ , 100 X 2.1mm. ID.
3. Mobile Phase: 15mM ammonium formate-Methanol (15:85 v/v)
4. Flow rate: 1.2 $\text{mL}\cdot\text{min}^{-1}$
5. Elution mode: Isocratic elution mode
6. Wavelength selected: 225nm
7. Temperature: Room temperature
8. Run time: 12 minutes
9. Retention time: Benidipine (1.22 min), Metoprolol (4.36 min)

System suitability tests for BEN and MET

System suitability test reveals the factors such as, theoretical plate (N), capacity factor (k'), resolution (R), separation factor (α), tailing factor (T), Mean \pm SD and RSD% and found to be in acceptable range for at least 6 successive injections of same analytes, as shown in Fig. 3 and Fig. 4. Table 1, represents the system suitability for BEN and MET.

Table 1: System suitability of BEN and MET

System suitability parameters	Benidipine (BEN)	Metoprolol (MET)	Acceptable Values
Theoretical plates (N)	189	709	> 2000
Capacity Factor (K')	3.786	4.563	> 1.5 - <10
Resolution (R)	---	6.26	≥ 2
Selectivity/Separation factor (α)	0.00	1.205	> k'
Asymmetry/Tailing factor (T)	1.8	1.8	> 2
Retention time (tR)	1.19 min.	4.32 min.	> k'
Wavelength of Detection (nm)	236 nm	225 nm	> 200 nm
Repeatability (%RSD)	1.88	1.65	< 2
Intra-Day Precision (%RSD)	1.12 - 2.15	0.25 - 1.78	< 2
Inter-Day Precision (%RSD)	0.82 - 2.04	0.25 - 1.12	< 2
Linearity range	32.5 - 500 $\mu\text{g}\cdot\text{ml}^{-1}$	32.5 - 500 $\mu\text{g}\cdot\text{ml}^{-1}$	NA
Regression equation	Y= 16744x - 83701	Y= 17885x + 102266	NA
SE of intercept (Se)	111428.4996	79653.06	NA
SD of intercept (Sa)	249161.6997	178109.67	NA
Correlation Coefficient (r^2)	0.998	0.9991	NA
LOQ ^a ($\mu\text{g}\cdot\text{mL}^{-1}$)	49.10 $\mu\text{g}\cdot\text{mL}^{-1}$	32.86 $\mu\text{g}\cdot\text{mL}^{-1}$	NA
LOD ^a ($\mu\text{g}\cdot\text{mL}^{-1}$)	148.80 $\mu\text{g}\cdot\text{mL}^{-1}$	99.58 $\mu\text{g}\cdot\text{mL}^{-1}$	NA

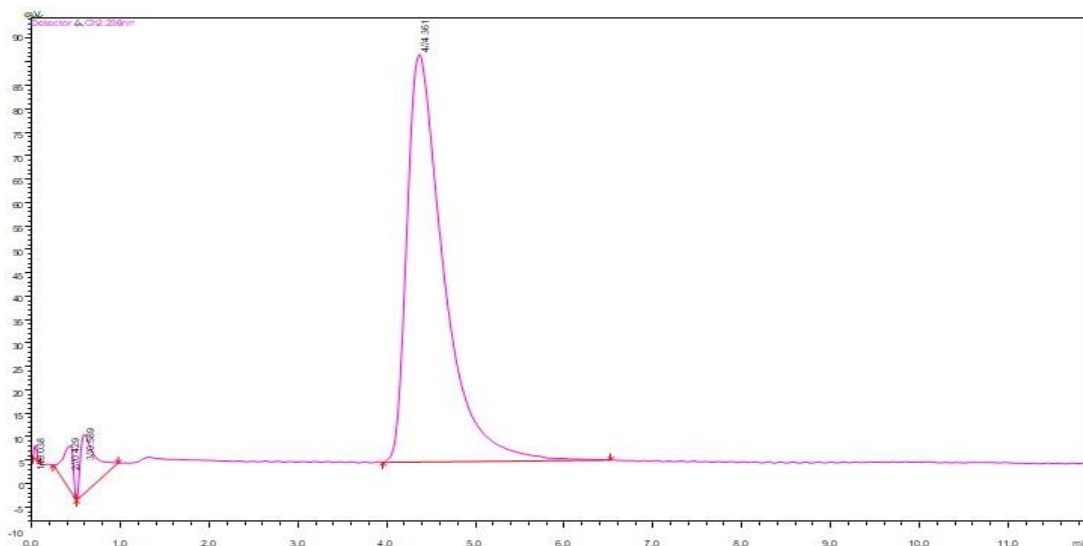


Figure 3: Chromatograph of MET (4.36 min) at flow rate 1.2 mL.min⁻¹

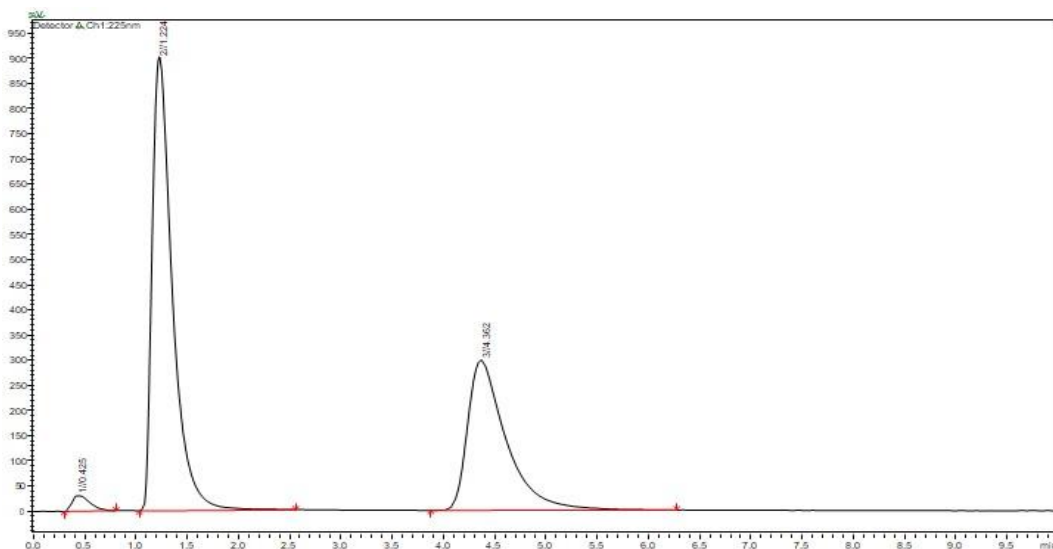


Figure 4: Chromatograph of BEN (1.22 min) and MET (4.36 min) for method development

Repeatability

Implementing the procedure mentioned, the homologous mixture of both BEN and MET of same concentrations

(500µg.mL⁻¹), were tested for six injections within the same day. The % RSD was calculated and found it is less than 2%; shown in (Table 2).

Table 2: Repeatability data of BEN and MET

Sr. No.	Benidepine		Metoprolol	
	Peak Area;	Conc. 250 ppm	Peak Area;	Conc. 250 ppm
1	12415863		7807488	
2	12463050		7820081	
3	12679087		7881049	
4	12669900		8012439	
5	12064694		7631657	
6	12635073		7929808	
Mean	12487944			7847087
STD. DEV.	235051.70			129649.49
RSD (%)	1.88			1.65

Intraday Precision:

Implementing the procedure mentioned, the homologous mixture of both BEN and MET of three replicates of three

different concentrations; 500 ppm, 250ppm and 125 ppm were tested and evaluated within the same day (intra-day precision). The %RSD was calculated and found less than 2%; shown in Table 3 and Table 4.

Table 3: Intraday Precision data of BEN

Drug Name: Benidipine (BEN)				
Sr. No.	Concentration (ppm)	Area	Mean \pm SD	%RSD
1	250 ppm	12415863	140348	1.12
	250 ppm	12463050		
	250 ppm	12679087		
2	250 ppm	12669900	224568.26	1.79
	250 ppm	12264694		
	250 ppm	12635073		
3	250 ppm	12249900	265995.45	2.15
	250 ppm	12124694		
	250 ppm	12315073		
Range of %RSD				1.12 – 2.15

Table 4: Intraday Precision data of MET

Drug Name: Metoprolol (MET)				
Sr. No.	Concentration (ppm)	Area	Mean \pm SD	% RSD
1	250 ppm	7807488	19921.100	0.25
	250 ppm	7820081		
	250 ppm	7781049		
2	250 ppm	8012439	47182.32	0.59
	250 ppm	7531657		
	250 ppm	7929808		
3	250 ppm	7881149	140492.86	1.78
	250 ppm	8012439		
	250 ppm	7731650		
Mean % RSD				0.25 – 1.78

Interday (intermediate) precision:

Implementing the procedure mentioned, the homologous mixture of both BEN and MET of three replicates of three different concentrations; 500 ppm, 250ppm and 125 ppm

were tested and evaluated in three successive days (interday/intermediate precision). The %RSD was calculated and found less than 2%; shown in Table 5 and Table 6.

Table 5: Interday (intermediate) Precision data of BEN

Drug Name: Benidipine (BEN)				
Sr. No.	Concentration (ppm)	Area	Mean \pm SD	% RSD
DAY 1	250 ppm	12615866	140766.44	1.12
	250 ppm	12403058		
	250 ppm	12669087		
DAY 2	250 ppm	12219900	99276.58	0.82
	250 ppm	12064694		
	250 ppm	12035055		
DAY 3	250 ppm	12269200	252006.03	2.04
	250 ppm	12111691		
	250 ppm	12605071		
Range of % RSD				0.82 – 2.04

Table 6: Interday (intermediate) Precision data of MET

Drug Name: Metoprolol (MET)				
Sr. No.	Concentration (ppm)	Area	Mean ± SD	% RSD
DAY 1	250 ppm	7807480	19921.10	0.25
	250 ppm	7820089		
	250 ppm	7781040		
DAY 2	250 ppm	8012439	54114.74	0.67
	250 ppm	8031657		
	250 ppm	7929808		
DAY 3	250 ppm	7929724	89067.80	1.12
	250 ppm	8012439		
	250 ppm	7834451		
Range of % RSD				0.25 – 1.12

Linearity

Under linearity or calibration studies, a linear relationship between area under peak values and selected drug concentration ($\mu\text{g.mL.min}^{-1}$) was plotted for five-six chosen concentrations of Benidipine (shown in Fig.5) and (shown in Fig.6). The regression equations, correlation coefficient

values (r), standard error of intercept (Se), standard deviation of intercept (Sa), limit of detection (LOD) and limit of quantification (LOQ) have been calculated. The linearity of the calibration curves was validated by the high value of correlation coefficient, acceptable values of regression coefficient, standard deviation of the slope and standard deviation of the intercept; shown in (Table 7 and Table 8).

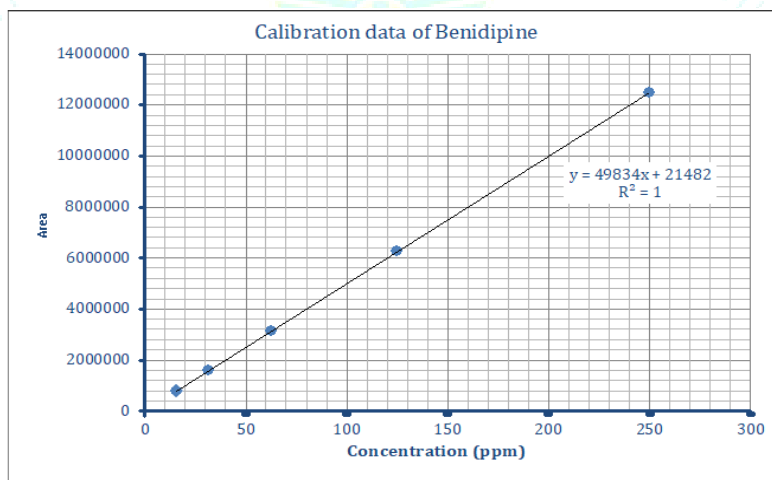


Figure 5: Calibration curve of Benidipine

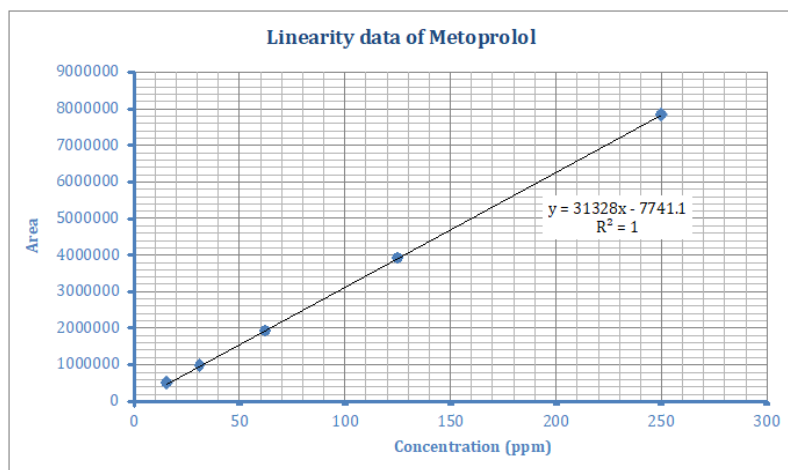


Figure 6: Calibration curve of Metoprolol

Table 7: Linearity data of Benidipine

Name of Drug; Benidipine			
Sr. No.	Concentration ($\mu\text{g.mL}^{-1}$)	Area	Average (Mean)
1	250 PPM	12415863	12439456
	250 PPM	12463050	
2	125 PPM	6227728	6231078
	125 PPM	6234428	
3	62.5 PPM	3119214	3109664
	62.5 PPM	3100114	
4	31.25 PPM	1552933	1553432
	31.25 PPM	1553931	
5	15.62 PPM	776466	774843
	15.62 PPM	773221	
6	Regression Equation		$Y = 49834x + 21482$
7	Correlation coefficient (R^2)		0.999
8	Std. Error of intercept		12200.22
9	Std. Dev. of intercept		27280.52
10	LOQ		1.80 ng.ml^{-1}
11	LOD		$5.47 \mu\text{g.ml}^{-1}$

Table 8: Linearity data of Metoprolol

Name of Drug; Metoprolol			
Sr. No.	Concentration ($\mu\text{g.mL}^{-1}$)	Area	Average (Mean)
1	250 PPM	7807488	7813784
	250 PPM	7820081	
2	125 PPM	3906802	3906802
	125 PPM	----	
3	62.5 PPM	1953477	1953477
	62.5 PPM	-----	
4	31.25 PPM	976724	976724
	31.25 PPM	-----	
5	15.62 PPM	488361	488361
	15.62 PPM	-----	
6	Regression Equation		$Y = 31328x - 7741.1$
7	Correlation coefficient (R^2)		1
8	Std. Error of intercept		79653.06
9	Std. Dev. of intercept		178109.67
10	LOD		14.28 ng.ml^{-1}
11	LOQ		g.ml^{-1}

Limit of detection (LOD/LOQ)

Limit of detection represents the concentration of analyte at S/N ratio of 3.3 and limit of quantification (LOQ) at which S/N is 10 were determined and results are given in Table 7 and Table 8. Low values of LOD and LOQ indicate sensitivity of the applied method for determination of mentioned drugs in tablets.

Robustness for the chromatographic method

The flow rate of the mobile phase was changed from 1 mL.min⁻¹ to 1.4 mL.min⁻¹; results was shown in Fig. 7 and Fig. 8 as well as in Table 9 and Table 10.

Similarly, the effect of deliberate changes in organic modifier (Methanol) composition was evaluated. In this study, the percentage composition of methanol was altered by $\pm 2\%$

(shown in Fig. 9 and Fig. 10) in the previous set of gradients to evaluate the effects on the separation behavior of BEN and MET. Finally, the wavelength (shown in Fig. 11 and Fig. 12) was changed by ± 2 nm wavelength and results were reported in Table 9 and Table 10.

From all above studies, after making deliberated changes in flow rate (± 0.2 mL.min⁻¹), organic modifier concentration; methanol ($\pm 2\%$) and wavelength (± 2 nm) have not made any significant changes in resolution, capacity factor and tailing factor. Nonetheless, it seems minute changes in robustness studies makes significant changes in theoretical plate counts. Robustness studies for BEN and MET displayed in Table 9 and Table 10.

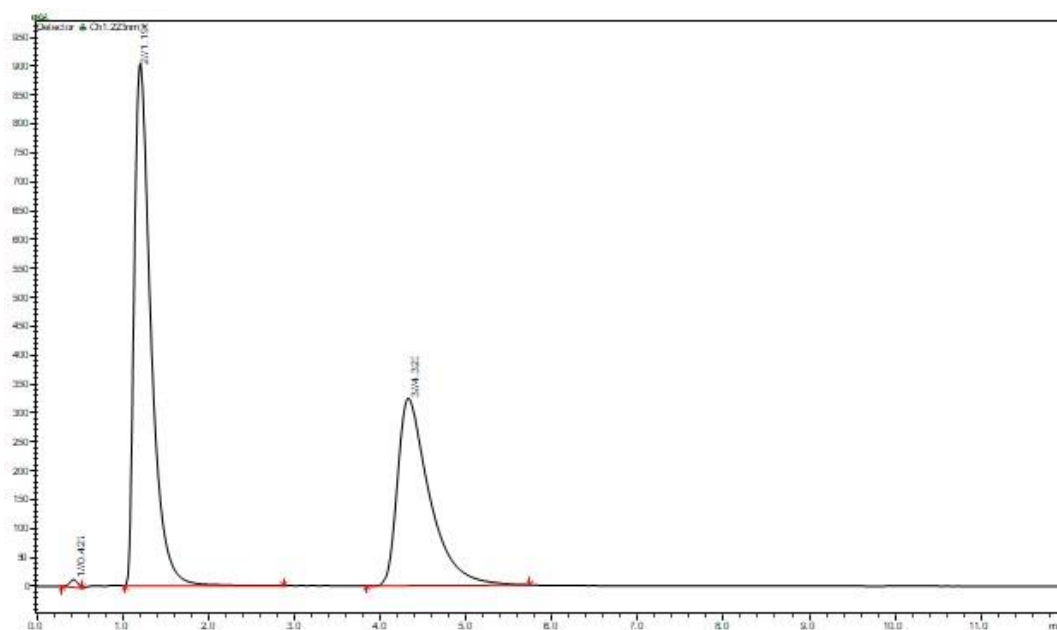


Figure 7: Chromatogram of BEN (1.19 min) and MET (4.32 min) at flow rate 1 mL.min⁻¹

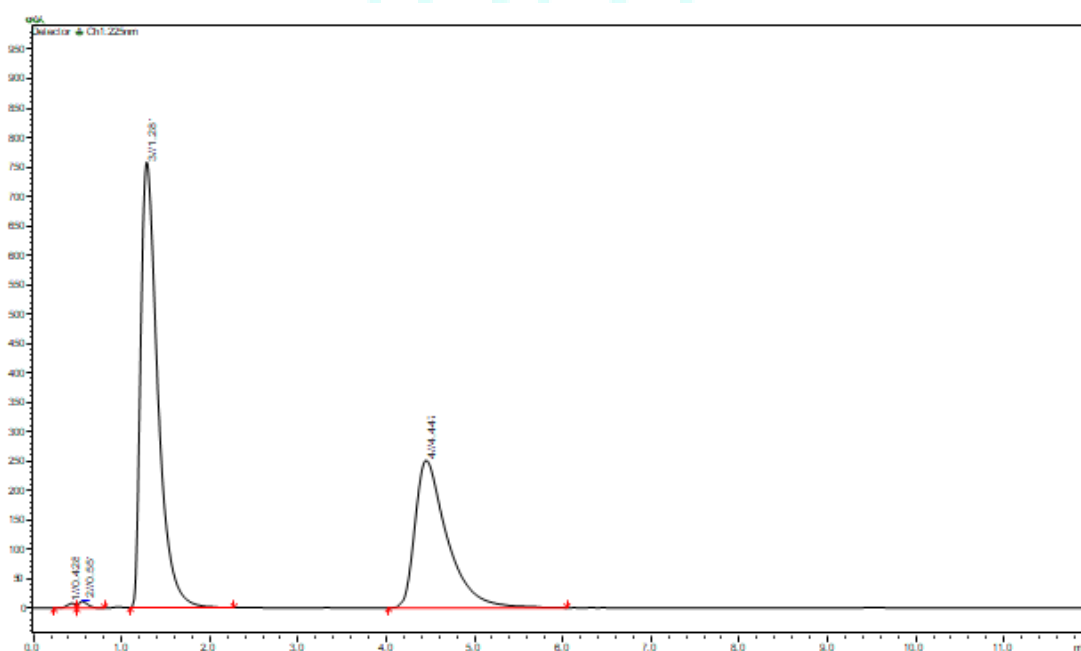


Figure 8: Chromatogram of BEN (1.28 min) and MET (4.44 min) depicts effects of flow rate 1.4 mL.min⁻¹

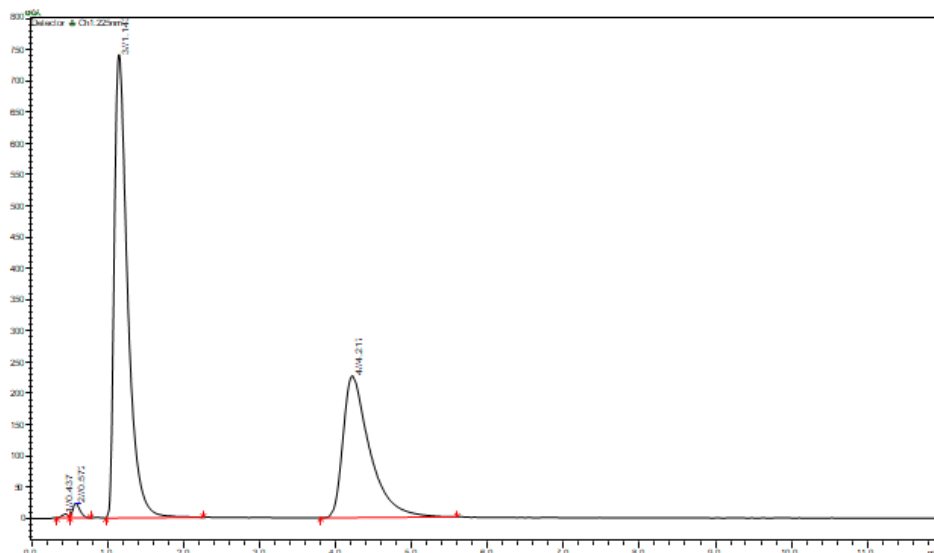


Figure 9: Robustness studies for BEN (1.14 min) and MET (4.42 min) at Methanol 73%

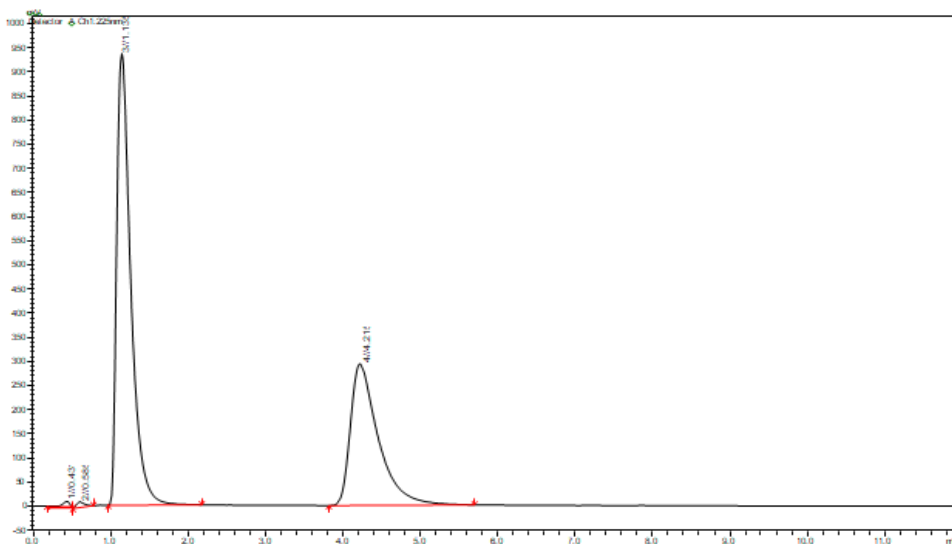


Figure 10: Robustness studies for BEN (1.33 min) and MET (4.21 min) at methanol 71%

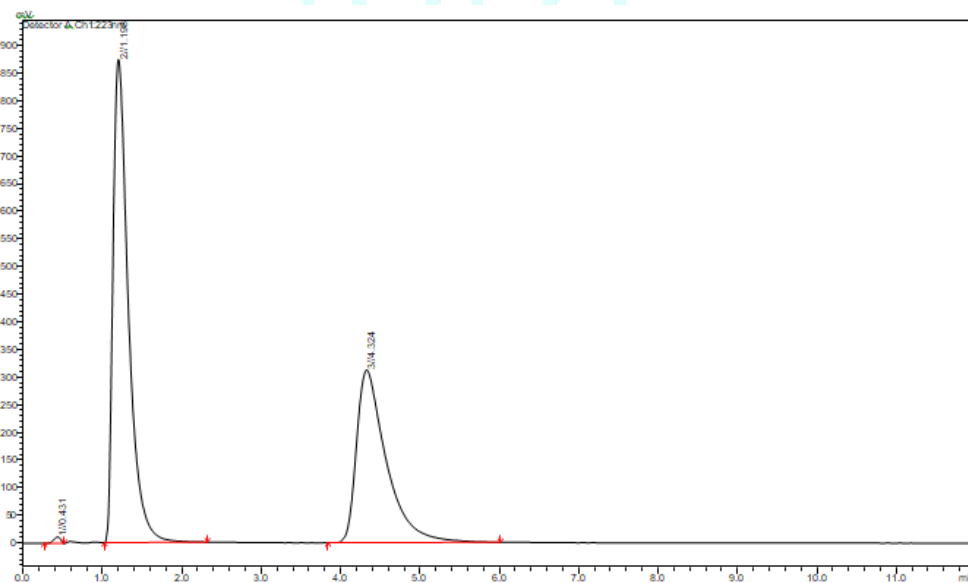


Figure 11: Robustness studies for BEN (1.19 min) and MET (4.32 min) at wavelength 223nm

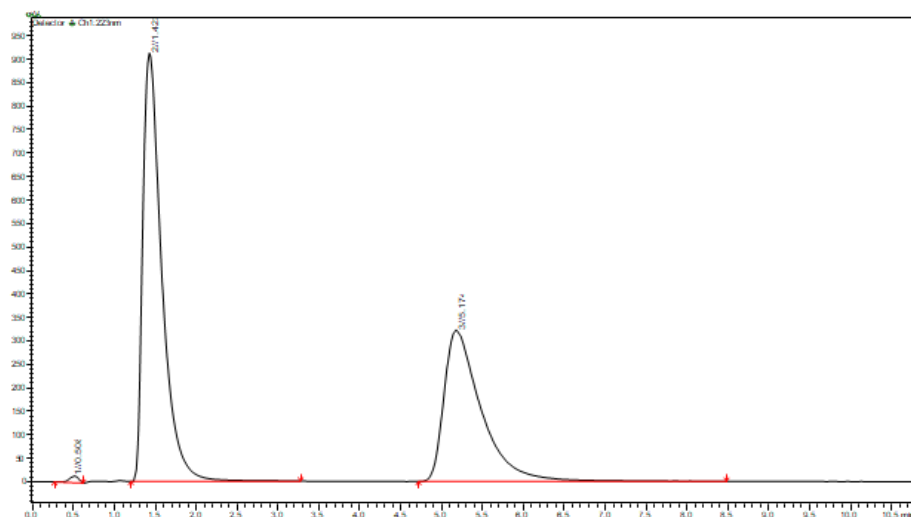


Figure 12: Robustness studies for BEN (1.42 min) and MET (5.17 min) at wavelength 225nm

Table 9: Robustness data of BEN

Sr. No.	F.(-0.2ml.mL ⁻¹)	F.(+0.2ml.mL ⁻¹)	A (-2ml)	A (+2ml)	WL (-2nm)	WL (+2 nm)
Resolution	----	----	----	----	----	----
Tailing factor	1.81	1.82	1.87	1.87	1.82	1.89
Capacity factor	1.21	1.89	1.99	1.19	1.83	1.88
Theoretical plates	710	785	802	791	819	832

Table 10: Robustness data of MET; calculated for resolution and tailing factor

Sr. No.	F.(-0.2ml.mL ⁻¹)	F(+0.2ml.mL ⁻¹)	A (-2ml)	A (+2ml)	WL (-2nm)	WL (+2nm)
Resolution	6.56	6.27	6.50	5.90	6.25	6.21
Tailing factor	1.88	1.83	1.82	1.80	1.86	1.88
Capacity factor	9.88	8.89	9.42	9.27	9.15	9.19
Theoretical Plates	625	722	218	782	827	867

Stability indicating method²⁷

Stability of both drugs are studied utilizing different parameter. In this study, the area of standard for stability and degradation of sample and standard were compare. Result shows BEN has highest degradation in oxidation and

acid as compare to others. MET did not showed degradation in oxidation, acid and basic environment. The standard area of BEN and MET as well as peaks of all parameters were given in Fig 13-16. The percent degradation of all parameters is given below in Tables 11 and 12.

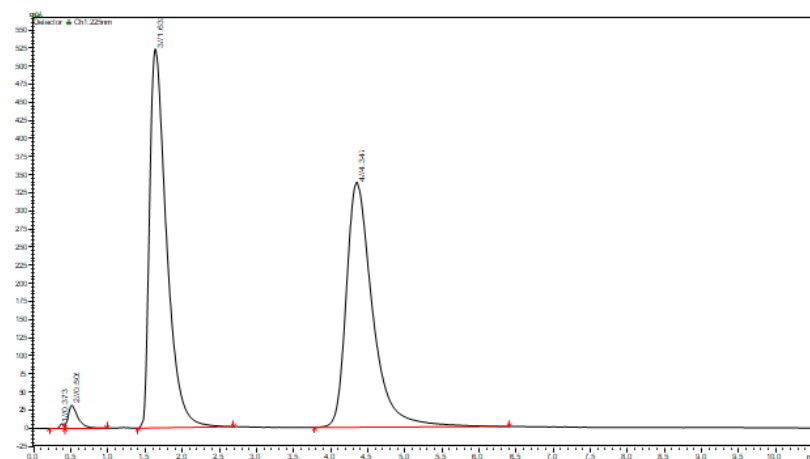


Figure 13: Force degradation data of BEN and MET at 50°C. (Neutral Hydrolysis)

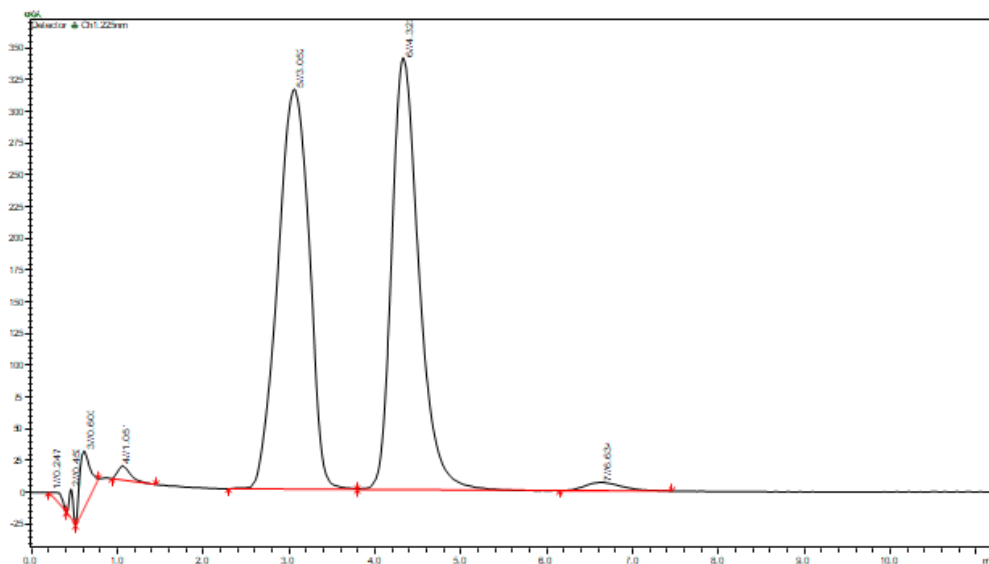


Figure 14: Force degradation data of BEN and MET at 0.1N HCl at 60°C

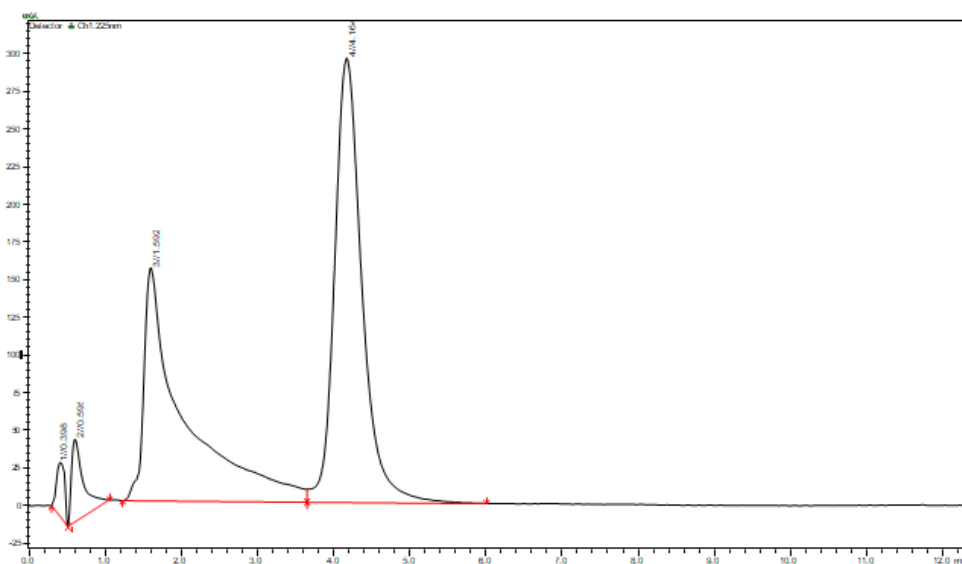


Figure 15: Force degradation data of BEN and MET at 0.1 N NaOH at 50°C.

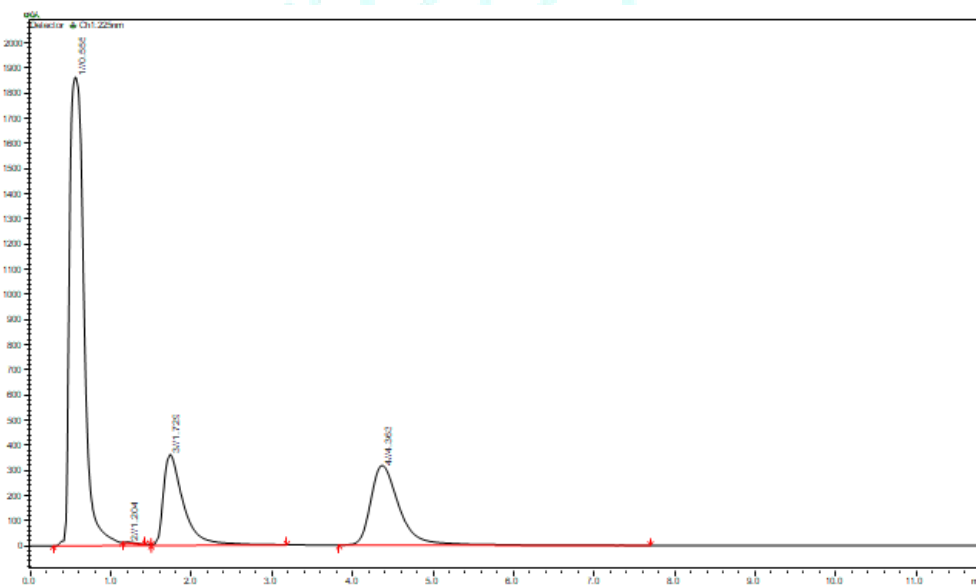


Figure 16: Force degradation data of BEN and MET at 3% H₂O₂ at room temperature

Table 11: Stability indicating studies of BEN

Conditions	Benidipine		Degradants of BEN
	% Area Std.	% degradation	No. of degradants
Acid (0.1N/M HCl) + 60°C + 12 Hrs.	88%	12%	1
Base (0.1N/M NaOH) + 60°C + 12 Hrs.	100%	0%	0
Thermal (60°C) + 12 Hrs.	100%	0%	0
Oxidation (3-6% H ₂ O ₂) + Room Temp.	47.44%	52.56	Not distinguished

Table 12: Stability indicating studies of MET

Conditions	Metoprolol		Degradants of MET
	% Area Std.	% degradation	No. of degradants
Acid (0.1N/M HCl) + 60°C + 12 Hrs.	100%	0%	0
Base (0.1N/M NaOH) + 60°C + 12 Hrs.	100%	0%	0
Thermal (60°C) + 12 Hrs.	100%	0%	0
Oxidation (3-6% H ₂ O ₂) + Room Temp.	100%	0%	0

4. CONCLUSION

From results and discussion, it has been concluded that the developed analytical method for the simultaneous estimation of benidipine (BED) and metoprolol (MET) in both bulk and tablet formulation has obliged the ICH guidelines. As per the ICH guidelines, the developed method has complied the linearity range (calibration data), drug recovery studies (%), repeatability, precision studies (intraday and interday/intermediate), and robustness. Moreover, as per the ICH guidelines, the system suitability test performed for simultaneous estimation of benidipine and metoprolol have achieved all guidelines; including, tailing factor (T), separation factors (α), theoretical plates (N), capacity factor (k'), resolution (R) and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid ascertained few degradation products for benidipine whereas the metoprolol was unaffected with forced degradation studies. Hence, this developed and validated method for simultaneous investigation by reverse phase high performance liquid chromatography can be used for routine analysis of estimation of both or either benidipine and metoprolol from marketed formulation.

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