

Estimate Hyoscine Butylbromide and Mefenamic Acid by RP-HPLC Method Development and Accelerated Stability Study in Pure and Combine Dosage Form

Jinal N. Tandel ^{1*}, Jatin Patel ¹, Rashmika R. Patel ², Samir K. Shah ³

¹ Department of Quality Assurance, Sardar Patel College of Pharmacy, Gujarat Technological University, Gujarat, INDIA

² Department of Pharmacognosy, Sardar Patel College of Pharmacy, Gujarat Technological University, Gujarat, INDIA

³ Department of Pharmacology, Sardar Patel College of Pharmacy, Gujarat Technological University, Gujarat, INDIA

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ABSTRACT

The aim of the present study was to develop and validate stability indicating HPLC method for simultaneous estimation of Hyoscoine butyl bromide (HBB) and Mefenamic acid (MEF). HPLC method for simultaneous analysis of both drugs was developed and validated according to ICH guideline. Efficient chromatographic separation was achieved on ODS column C₁₈ (250 mm × 4.6 mm, 5 μm) using the optimized mobile phase. Stability indicating assay method was carried out by different stress degradation conditions. In HPLC method, the Retention time for HBB and MEF was 3.21 and 5.07 min using optimized mobile phase potassium dihydrogen phosphate buffer (pH 5.0) and methanol (60:40 % v/v) with a flow rate of 1 ml/min. The multiple wavelength UV detector was set at a 237 nm for measurement of all compound. Quantification based on measuring the peak areas. The degradation of HBB, MEF and Formulation was shown to be highest in alkaline condition. Linearity was observed in concentration range of 1- 3μg/ml and 12.5-37.5 μg/ml for HBB and MEF respectively. All validation parameters were within the acceptable range. Moreover, the % RSD for repeatability, inter and intraday precision was found to be within the range, which reveals that the method is precise. Accuracy study of the drug in marketed preparation also report in the limit. Assay of the dosage form finalized the applicability of this method for estimation of Hyoscine Butylbromide and Mefenamic Acid tablet dosage form.

Keywords: Mefenamic Acid (MEF), Hyoscine Butylbromide (HBB), RP-HPLC, stability indicating method, validation

INTRODUCTION

Hyoscine Butylbromide (HBB) is a Peripherally acting anti muscarinic, anticholinergic, agent. It is used in pain and discomfort caused by menstrual cramps, abdominal pain, or other spasmodic activity [1]. Chemically it is (1S,3s,5R,6R,7S,8r)-6,7-epoxy-8-butyl-3 [(S)Tropoyloxy] tropanium Bromide (**Figure 1**) [2].

Mefenamic acid is a non steroidal anti-inflammatory agent with analgesic, anti inflammatory and anti pyretic properties, it is an inhibitor of cycloxygenase [3]. Chemically it is 2-[(2.3-Dimethyl phenyl) amino] benzoic acid [4].

The combination formulation is used as an anti spasmodic analgesic for the spasm in GIT and UTI. Literature study reveals that there are number of method published for the HBB alone and in combination with other drugs like UV [5-7], HPLC [8,9], stability indicating HPLC [10], LC-MS [11]. For MEF UV [12,13], HPLC [14-17], HPTLC [18], stability indicating HPLC [19,20] are reported in single and in combination with other drugs. There are no any single method was found for the estimation of these two drugs in combination. Both drugs are official in IP-2014 [21], BP-2009 [22], USP30-NF25 [23]. The present work, a successful attempt has been made to estimate both these drugs simultaneously using stability indicating RP- HPLC method in different conditions. This study attempts to

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✉ Jinaltandel1202@gmail.com (*Correspondence) ✉ jatinp928@gmail.com ✉ rashmikacpatel@gmail.com

✉ mrsamirshah@gmail.com

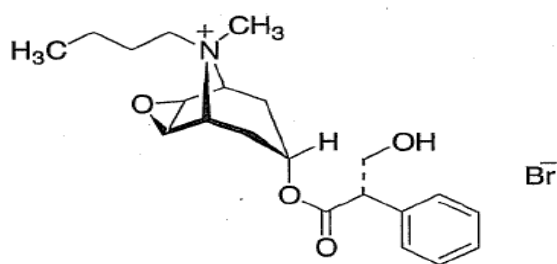


Figure 1. Chemical Structure of Hyoscine Butylbromide

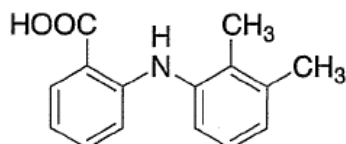


Figure 2. Chemical Structure of Mefenamic acid

develop a simple, accurate and precise analytical chromatographic method, which can quantify these drugs simultaneously from a combined tablet dosage form. The developed method was validated as per ICH guidelines and found to comply with the acceptance Criteria [24,25].

EXPERIMENTAL SECTION

Materials and Reagents

MEF was procured from Triveni Interchem Pvt Ltd Vapi and HBB was procured from Sovereign Pharma Pvt Ltd Daman as gift sample. All the reagents of HPLC grade including Water, Methanol, Acetonitrile were procured from Finar. NaOH, H₂O₂, dihydrogen phosphate and HCl were procured from Spectrochem. The Pharmaceutical formulation used in this study was Hyoscimax-MF Tablet procured from the local market and labeled to contain 20mg HBB and 250 mg MEF per Tablet.

Instrumentation

Analytical Technologies HPLC System consist of S-1122 Solvent delivery system (pump), 2203 UV- Visible detector, Rheodyne injector with 20 µl loop injector. Alchrome A -2000 Software system controller. Whatman filter paper no. 41, pH meter Systronics model no. 335 were used. A reverse phase C₁₈ (250 mm× 4.6 mm, 5 µm) analytical column was used. Weighing was done on Swissler.

Preparation of Standard Solutions

Preparation of mobile phase

Composition: Potassium dihydrogen phosphate buffer (pH 5.0): Methanol (60:40 v/v)

Preparation of Buffer: Take 6.8 gm of Potassium dihydrogen phosphate buffer and add into the 800 ml of water and shake well to dissolve the KH₂PO₄ and then add 200 ml of water to make 1000 ml of Buffer Solution. Adjust the pH of Buffer with the 0.1 N NaOH to make the pH of Buffer 5.0

MEF standard stock solution: (250 µg/ml)

A 250 mg of MEF was weighed and transferred to a 100 ml volumetric flask and volume made up by methanol (2500 µg/ml). Take 10 ml from this solution in 100 ml volumetric flask and volume was made up to the mark with methanol.

HBB standard stock solution: (20 µg/ml)

A 200 mg of HBB was weighed and transferred to a 100 ml volumetric flask and volume was made up to the mark with methanol (2000 µg/ml). Take 10 ml from this solution in 100 ml volumetric flask and make up with

methanol (200 µg/ml). From the above solution take 10 ml in 100 ml volumetric flask and make up the volume with methanol.

Preparation of standard solution of binary mixtures of MEF (25 µg/ml) and HBB (2 µg/ml)

Take 1 ml from the MEF stock solution and 1ml from HBB stock solution and transferred to 10 ml volumetric flask and volume made up to the mark by methanol.

Preparation of Sample Solution

Take Tablet Powder equivalent to 250 mg of MEF and 20 mg of HBB was transferred to a 100 ml volumetric flask and make up 25 ml with Mobile phase shake well and Sonicate for 15 minutes and finally make up the volume up to 100 ml. The solution was filtered through Whatman filter paper no.42. Take 1 ml from this and transferred to 10 ml volumetric flask and made up volume up to the mark with mobile phase. [MEF 25 µg/ml and HBB 2 µg/ml] The solution was injected 20 µl. The areas of resulting peak were measured at 237nm.

Selection of Elution Mode

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also better performing in terms of efficiency, stability and reproducibility. C₁₈ column is least polar compare to C₄ and C₈ columns. Here, A 250 x 4.6 mm column of 5.0µm particle packing was selected for separation of MEF and HBB. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability.

Selection of Wavelength

Standard solution of MEF (25 µg/ml) and Standard solution of HBB (2 µg/ml) were scanned between 200-400 nm using UV-visible spectrophotometer. Both solutions were scanned between 200-400 nm. Wavelength was selected from the overlay spectra of above solutions.

System Suitability Test

It is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

Formulas for calculation of SST are,

Resolution

$$R_s = \frac{tR_2 - tR_1}{0.5(w_1 + w_2)}$$

where,

R_s is resolution

tR_1 and tR_2 are the retention times of components 1 and 2,

w_1 and w_2 are peak width of components 1 and 2.

Theoretical plate

$$N = 16 \left(\frac{tR}{w} \right)^2$$

where,

N is no of theoretical plate.

tR is the retention time.

w is the peak width

Tailing factor

$$T = \frac{W_{0.05}}{2f}$$

where,

T is tailing factor

*W*_{0.05} is width of peak at 5% height and *f* is distance at 5% height.

Chromatographic Conditions

- ✓ **Column:** C₁₈ (250 mm × 4.6 mm, 5µm) Hypersil BDS
- ✓ **Mobile Phase:** Phosphate Buffer (pH 5): Methanol (60:40 % v/v)
- ✓ **Flow Rate :** 1.0 ml/min
- ✓ **Detection Wavelength:** 237 nm
- ✓ **Runtime:** 10 min
- ✓ **Injection volume:** 20.0 µl

Stability Indicating Method

Acid degradation

Acid decomposition studies were performed by Transferring 1 ml of stock solution in to 10 ml of volumetric flask. 2 ml of 0.1 N HCl solutions was added and mixed well and put for 4 hrs. After time period the volume was adjusted with diluent to get 2 µg/ml for HBB and 25 µg/ml for MEF.

Base degradation

Basic decomposition studies were performed by Transferring 1 ml of stock solution in to 10 ml of volumetric flask. 2 ml of 0.1 N NaOH solutions was added and mixed well and put for 4 hrs. After time period the volume was adjusted with diluents to get 2 µg/ml for HBB and 25 µg/ml for MEF.

Oxidative degradation

Oxidative decomposition studies were performed by Transferring 1 ml of stock solution in to 10 ml of volumetric flask. 2 ml of 3% H₂O₂ solutions was added and mixed well and put for 4 hrs. After time period the volume was adjusted with diluent to get 2 µg/ml for HBB and 25 µg/ml for MEF.

Photo degradation

Photo Degradation studies were performed by Transferring 1 ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was keep in presence of Sunlight for 48 h. Then the volume was adjusted with diluent to get 2 µg/ml for HBB and 25 µg/ml for MEF.

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 110°C for 4 h. Then the volume was adjusted with diluent to get 2 µg/ml for HBB and 25 µg/ml for MEF.

Validation of RP-HPLC Method

Linearity

The linearity for MEF and HBB were assessed by analysis of combined standard solution in range of 12.5-37.5µg/ml and 1-3 µg/ml respectively. 5, 7.5, 10, 12.5,15 solutions were pipette out from the Stock solution of MEF (250 µg/ml) and HBB (2 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 12.5,18.75,25,31.25 and 37.5 µg/ml and 1,1.5,2,2.5 and 3 µg/ml for MEF and HBB respectively

In term of slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted.

Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

A. Repeatability

Middle concentration from calibration curve of MEF and HBB was injected six times and areas of peaks were measured and %R.S.D. was calculated.

B. Intra-day precision

Standard solution containing lower, middle, higher concentration range of MEF and HBB were analyzed three times on the same day and %R.S.D was calculated.

C. Inter-day precision

Standard solution containing lower, middle, higher concentration range of MEF and HBB were analyzed three times on the different day and %R.S.D was calculated.

Accuracy

✓ For MEF

17 µg/ml drug solution was taken in three different flasks label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 100ml. The area of each solution peak was measured at 237 nm. The amount of MEF was calculated at each level and % recoveries were computed.

✓ For HBB

1.3 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 100ml. The area of each solution peak was measured at 237 nm. The amount of HBB was calculated at each level and % recoveries were computed.

LOD and LOQ

The LOD was estimated from the set of 6 calibration curves used to determination method linearity. The LOD may be calculated as,

$$LOD = 3.3 \times (SD/Slope)$$

where,

SD= Standard deviation of Y-intercepts of 6 calibration curves.

Slope = Mean slope of the 6 calibration curves.

The LOQ was estimated from the set of 6 calibration curves used to determine method linearity. The LOQ may be calculated as,

$$LOQ = 10 \times (SD/Slope)$$

where,

SD = Standard deviation of Y-intercepts of 6 calibration curves.

Slope = Mean slope of the 6 calibration curves.

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 4.8 and 5.2.
3. Ratio of Mobile phase was changed (± 2) Buffer: Methanol (58:42) and Buffer: Methanol (62:38)

Analysis of marketed formulation by developed method

Take Tablet Powder equivalent to 250 mg of MEF and 20mg of HBB was transferred to a 50ml volumetric flask and make up 25 ml with Mobile phase shake well and Sonicate for 15 minutes and finally make up the volume up to 50 ml. The solution was filtered through Whatman filter paper no.42. Take 1 ml from this and transferred to 10 ml volumetric flask and made up volume up to the mark with mobile phase. [MEF 25 µg/ml and HBB 2 µg/ml] The solution was injected 20 µl. The areas of resulting peak were measured at 237 nm.

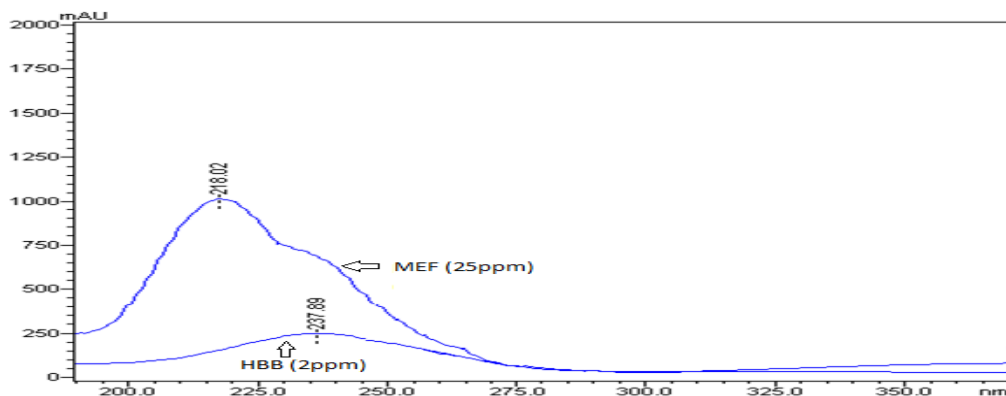


Figure 3. Overlay UV Spectrum of MEF and HBB showing selection of wavelength detection

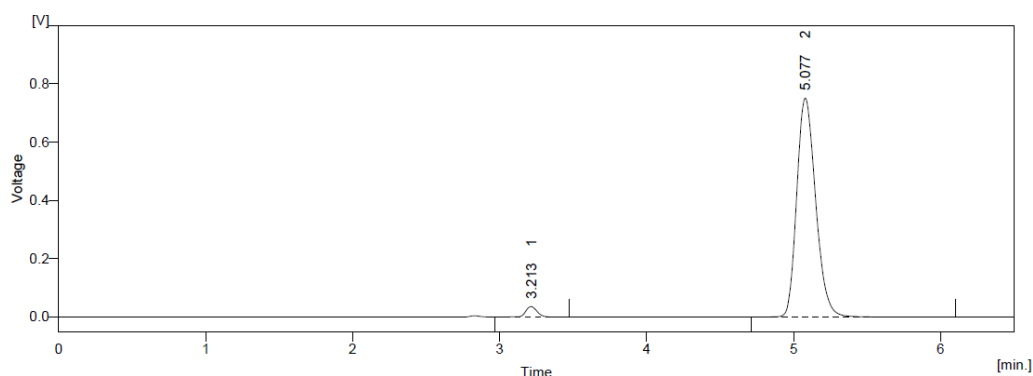


Figure 4. Chromatogram of MEF- HBB in Buffer (pH 5.0):Methanol (60:40 % v/v)

Table 1. Results for system suitability test

Parameters	Data observed	
	HBB \pm SD	MEF \pm SD
Retention time (min.)	3.213 \pm 0.14	5.077 \pm 0.12
Theoretical plates per column	8237 \pm 91.23	7284 \pm 44.25
Symmetry factor/Tailing factor	1.300 \pm 0.8	1.438 \pm 0.15
Resolution	9.819 \pm 0.23	

RESULT AND DISCUSSION

Selection of Wavelength

Both MEF and HBB show reasonably good response at 237nm. Shown in [Figure 3](#).

Selection of Mobile Phase

After considering the varying combinations of various mobile phases, Phosphate Buffer: Methanol (60:40 % v/v pH 5). Take 6.8 gm KH_2PO_4 into a 1000 ml beaker, add 800 ml water and dissolve it adjust pH 5 with OPA. Make up Volume 1000 ml with water was finalized as it was showing good peak shapes and a significant amount of resolution. Final chromatogram of HBB and MEF are shown in [Figure 4](#).

System suitability Parameters- Result of system suitability test are shown in [Table 1](#).

Stability-Indicating Method

Acid degradation

Acid decomposition studies were performed with 2 $\mu\text{g}/\text{ml}$ of HBB and 25 $\mu\text{g}/\text{ml}$ of MEF. Chromatogram for the studies were given in [Figures 5-8](#).

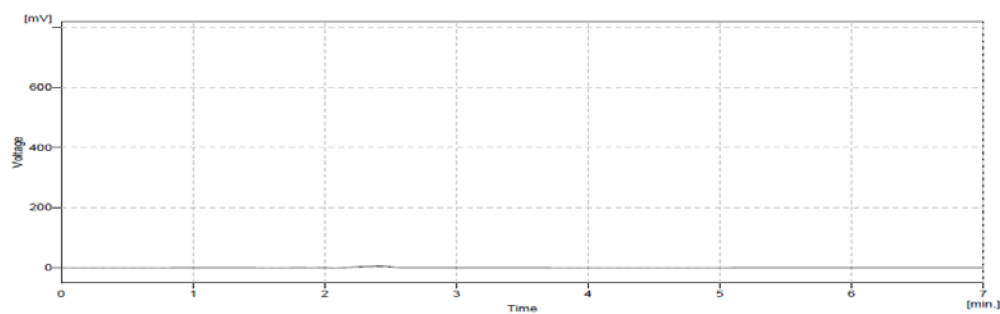


Figure 5. Acid Degradation Blank

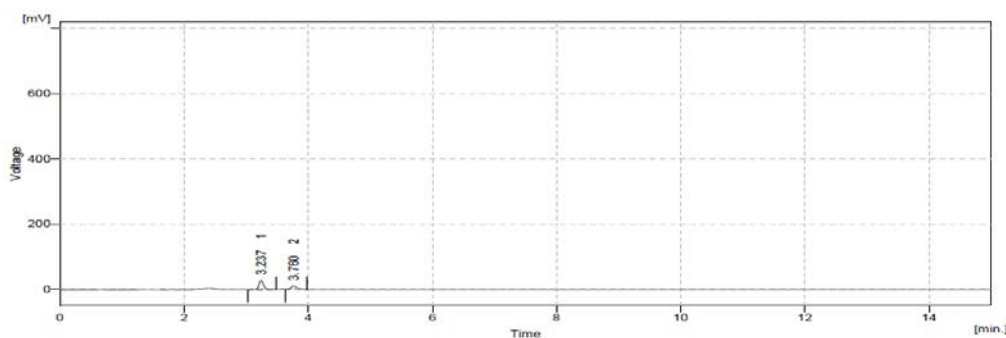


Figure 6. HBB Acid Degradation Standard

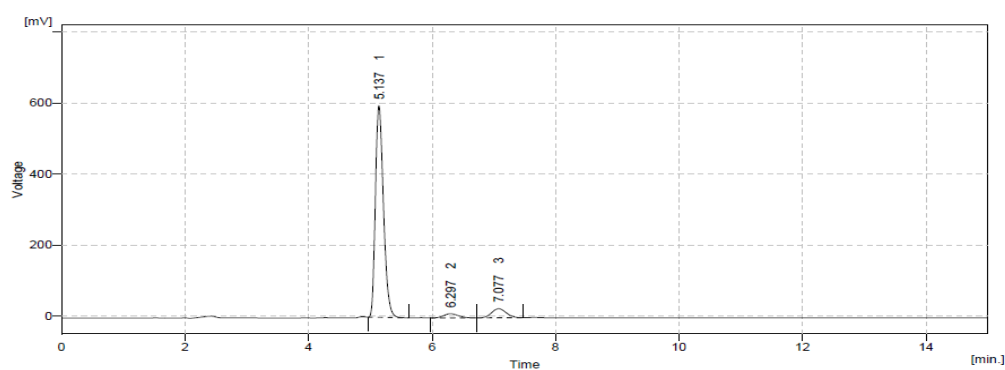


Figure 7. MEF Acid Degradation Standard

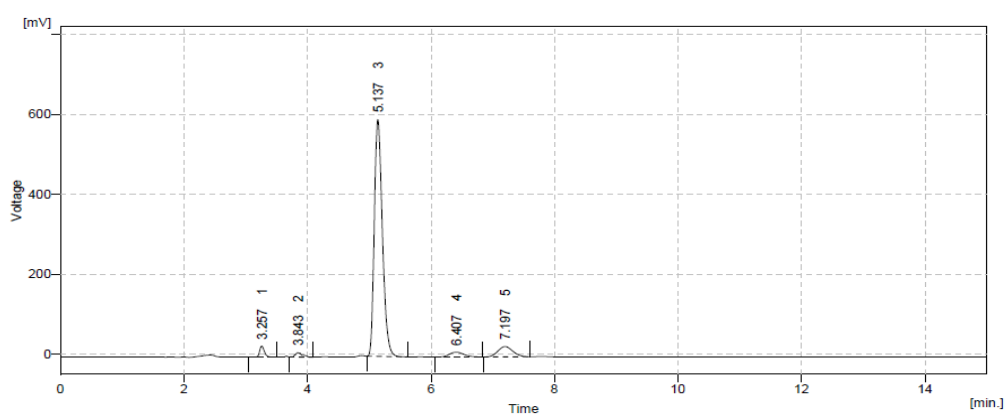


Figure 8. HBB and MEF Acid Degradation Sample

Base degradation

Basic decomposition studies were performed with 2 $\mu\text{g/ml}$ of HBB and 25 $\mu\text{g/ml}$ of MEF. Chromatogram for degradation were shown in [Figures 9-12](#).

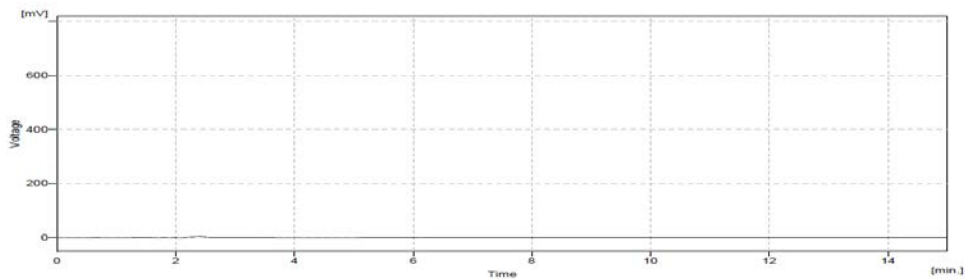


Figure 9. Base Degradation Blank

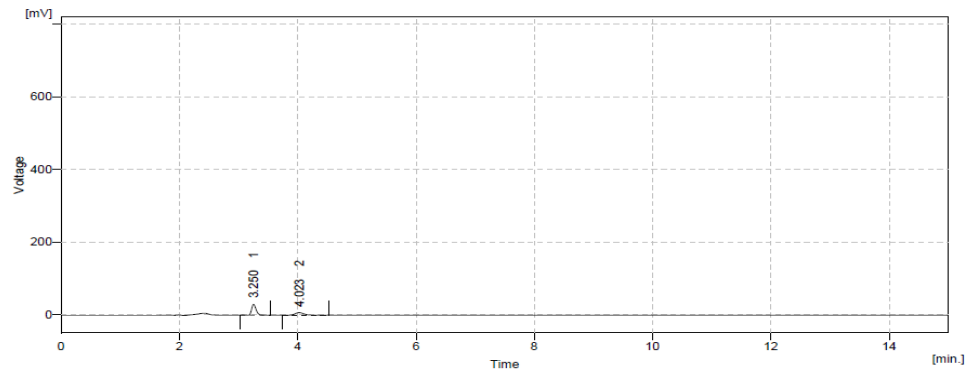


Figure 10. HBB Base Degradation

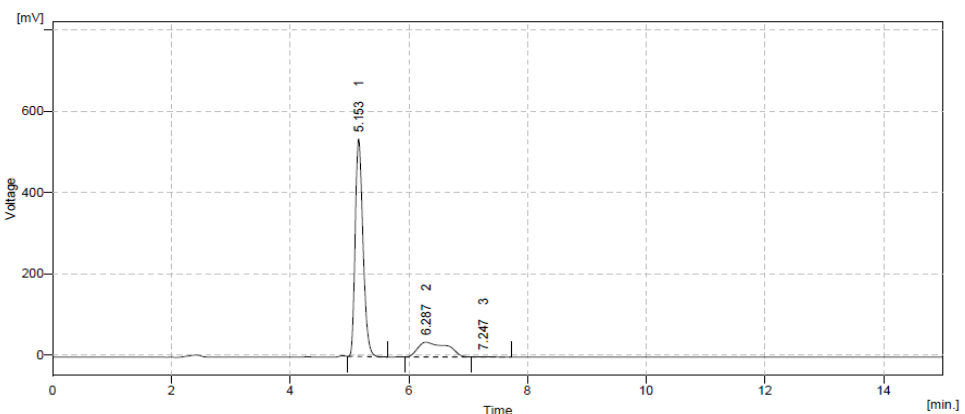


Figure 11. MEF Base Degradation

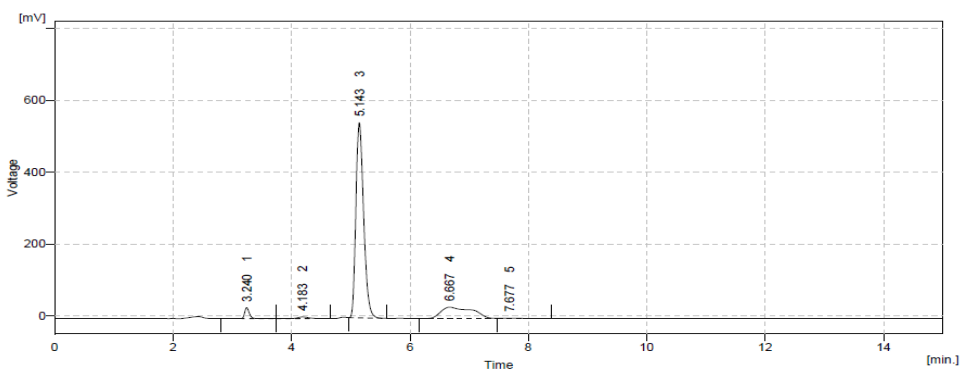


Figure 12. HBB and MEF Base Degradation Sample

Oxidative degradation

Oxidative decomposition studies were with 2 µg/ml for HBB and 25 µg/ml for MEF. Chromatogram for degradation were shown in [Figures 13-16](#).

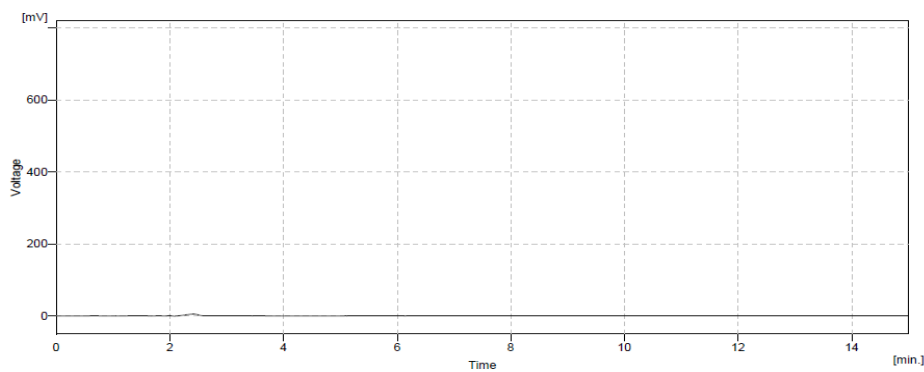


Figure 13. Oxidation Degradation Blank

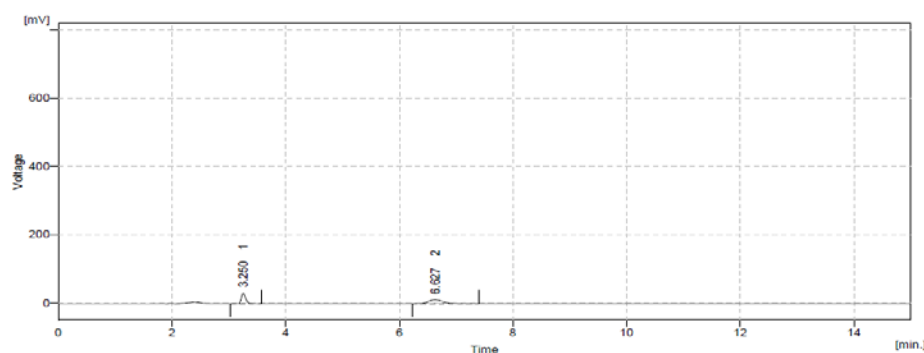


Figure 14. HBB Oxidation Degradation

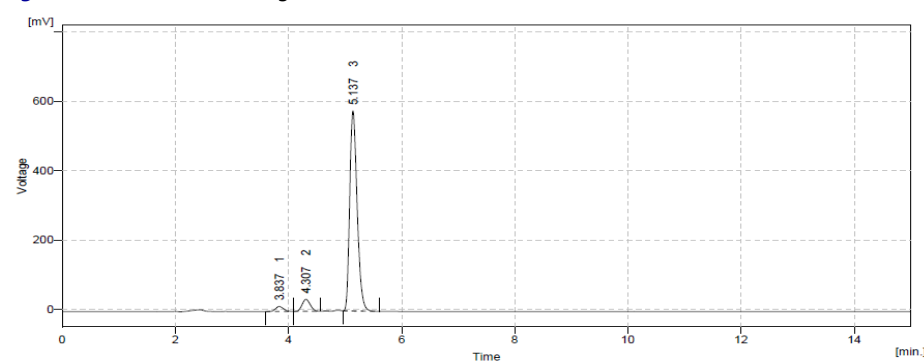


Figure 15. MEF Oxidation Degradation

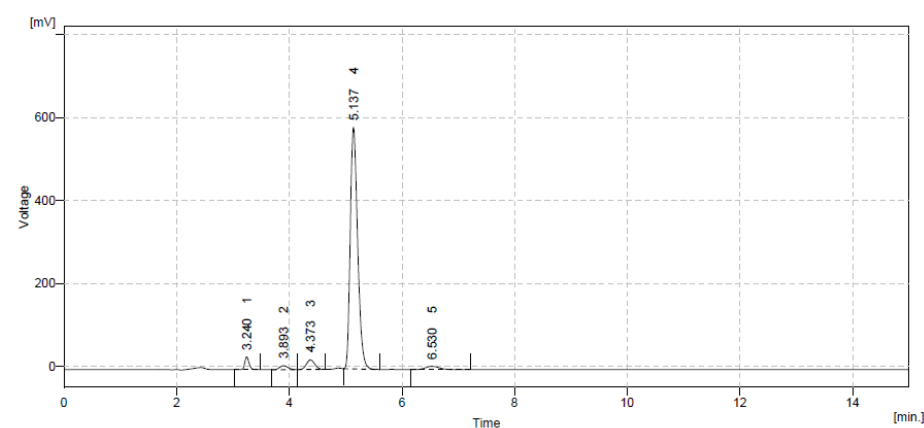


Figure 16. HBB and MEF Oxidation Degradation sample

Photo degradation

Photo Degradation studies were performed with 2 $\mu\text{g}/\text{ml}$ for HBB and 25 $\mu\text{g}/\text{ml}$ for MEF. Chromatogram for degradation were shown in **Figures 17-20**.

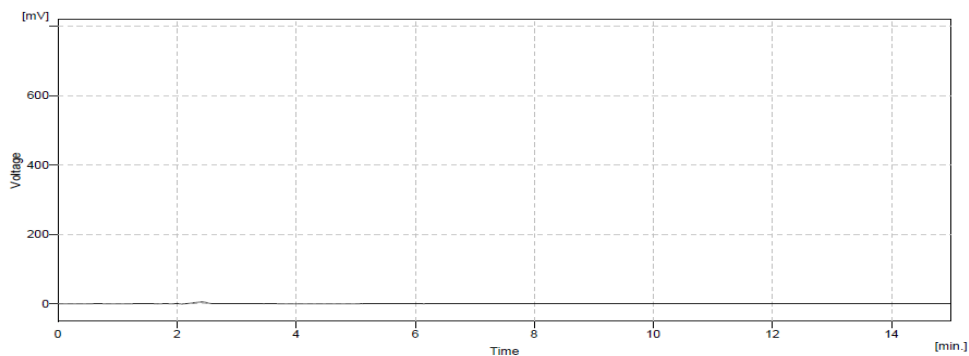


Figure 17. Photo Degradation Blank

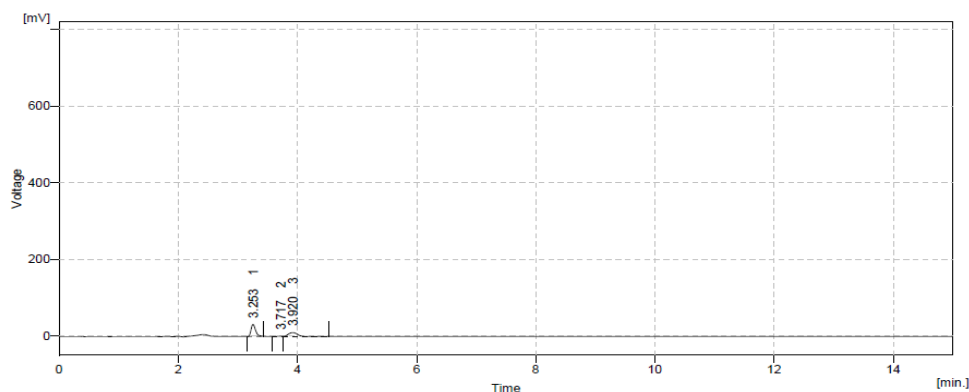


Figure 18. HBB Photo Degradation

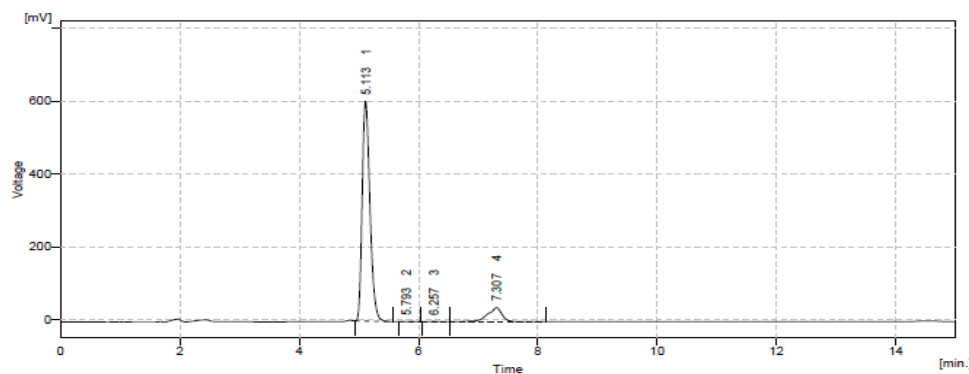


Figure 19. MEF Photo Degradation

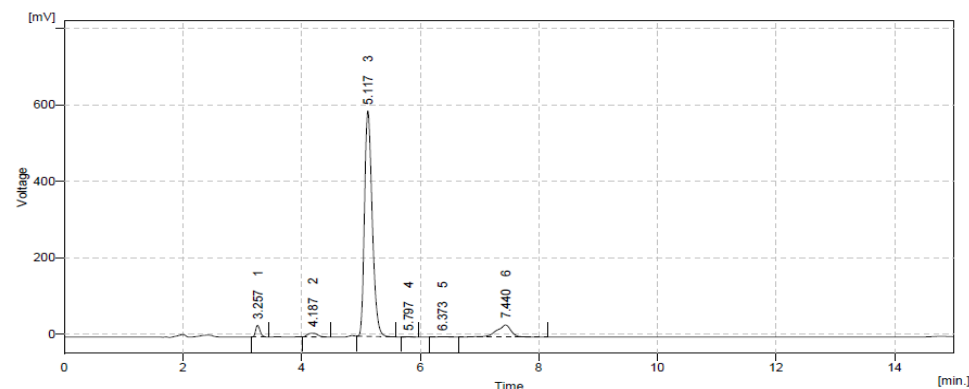


Figure 20. HBB and MEF Photo Degradation sample

Thermal degradation

Thermal Degradation studies were performed with 2 µg/ml for HBB and 25 µg/ml for MEF. Chromatogram for degradation were shown in [Figures 21-24](#).

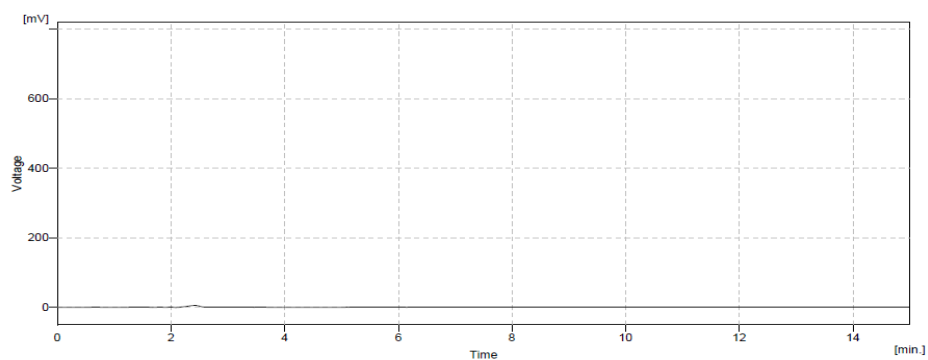


Figure 21. Thermal Degradation Blank

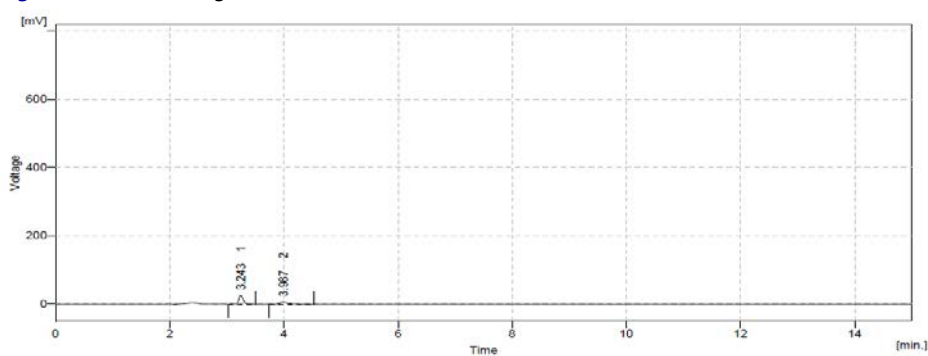


Figure 22. HBB Thermal Degradation

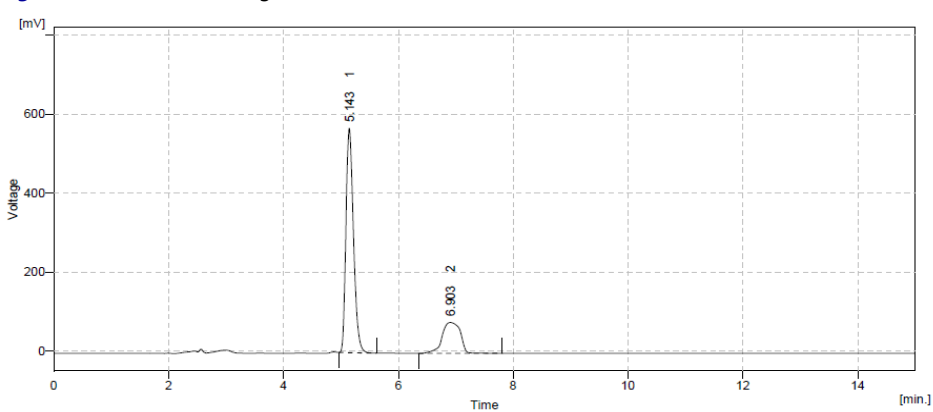


Figure 23. MEF Thermal Degradation

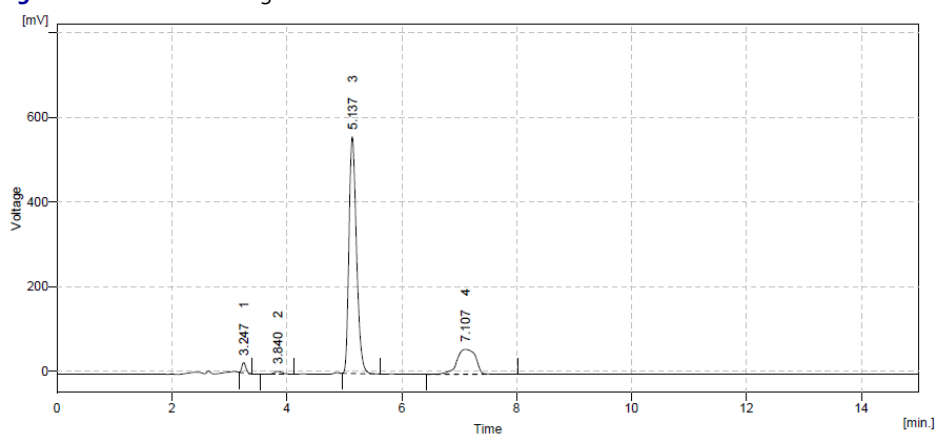


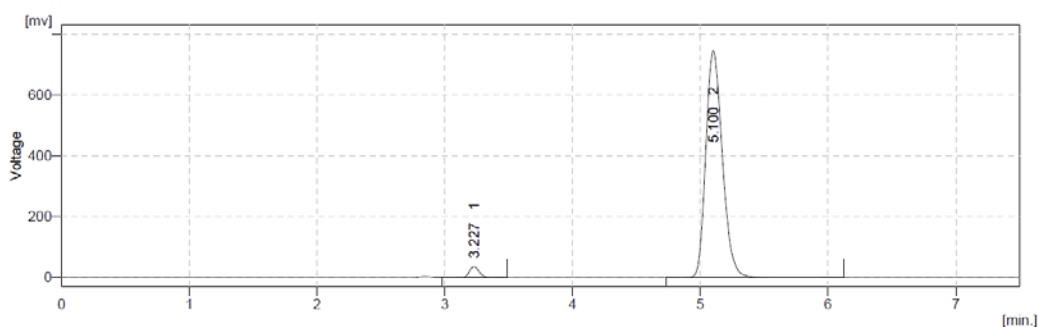
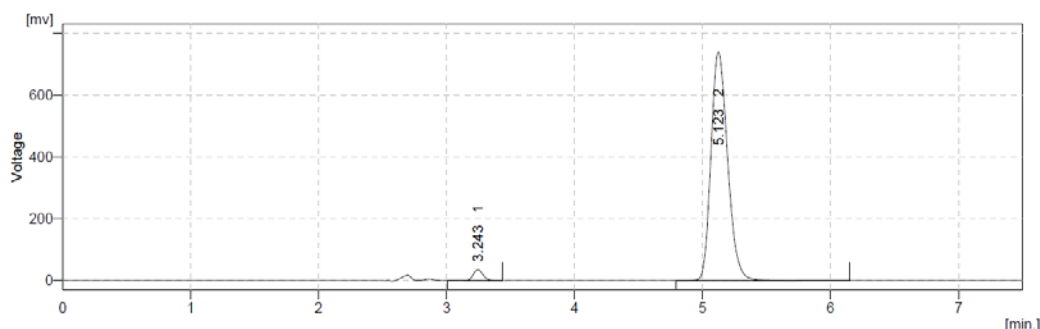
Figure 24. HBB and MEF Thermal Degradation sample

Result for the % Degradation

Data for the %Degradation of HBB and MEF in standard as well as in sample was shown in [Table 2](#).

Table 2. % Degradation of HBB and MEF

Parameter	HBB		MEF	
	Standard	Sample	Standard	Sample
	%Degradation		%Degradation	
Acid	18.23	18.92	12.74	12.16
Base	16.82	16.34	18.33	18.97
Thermal	20.06	20.78	15.55	16.09
Oxidation	15.56	15.08	13.02	14.05
Photo	11.42	11.01	17.43	17.90

**Figure 25.** Chromatogram of MEF and HBB std**Figure 26.** Chromatogram of MEF and HBB sample

VALIDATION

Specificity of the drugs were given by standard and sample of HBB and MEF shown in (Figure 25-27) Linearity graph for the both drugs are shown in (Figure 28) Calibration graphs were constructed by plotting the peak area versus their corresponding concentrations (Figure 29 and 30). Good linearity was obtained in the range of 12.5-37.5 μ g/ml and 1-3 μ g/ml for MEF and HBB respectively. The results are shown in Table 3 and 4. LOD and LOQ were calculated from the slope and standard deviation of y-intercepts of the regression line of the calibration curve. The results are shown in Table 5. The precision of the method and instrument precision was evaluated and relative standard deviation (RSD) values were calculated. The RSD values for MEF and HBB showed that the precision of the method was satisfactory. The results are shown in Table 5. The accuracy of the method was determined by recovery studies. The recoveries were close to 100% for MEF and HBB. The results are shown in Table 6. Developed method was found to be robust when the mobile phase ratio, flow rate and pH was changed. The results are shown in Table 7 and 8.

Specificity

See Figures 25-30 and Tables 3-9.

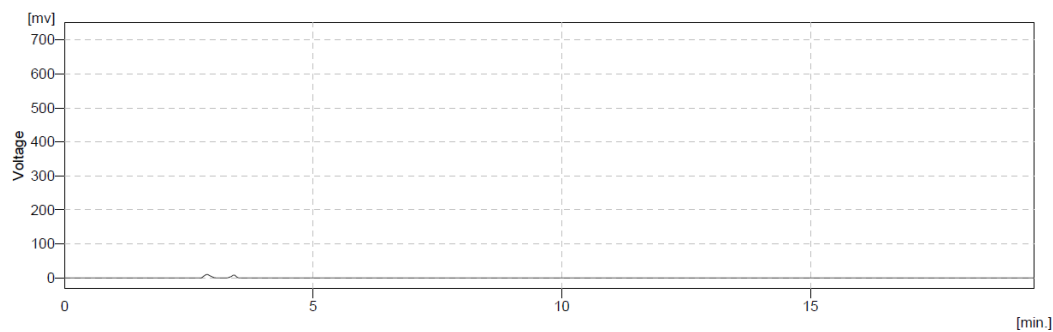


Figure 27. Chromatogram of Blank

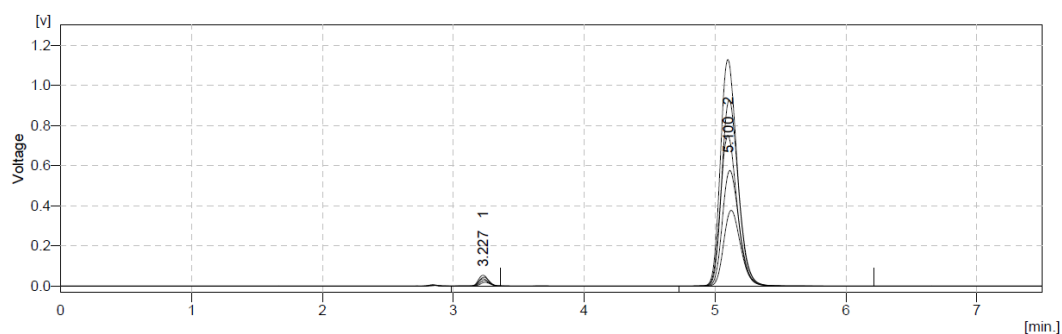


Figure 28. Overlay chromatogram of different concentrations of binary mixtures of MEF and HBB

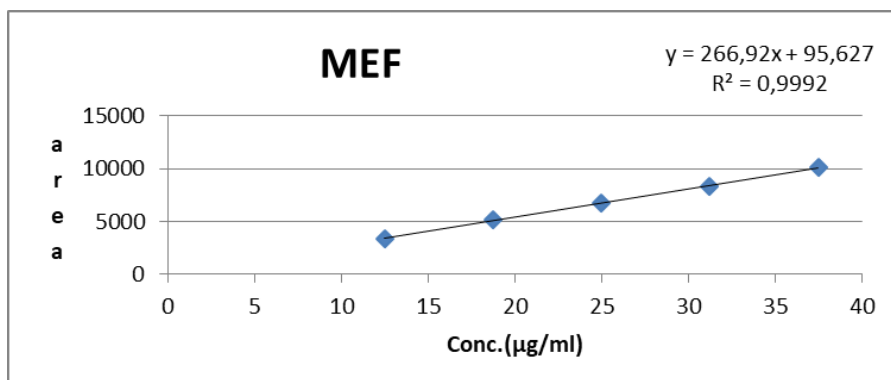


Figure 29. Calibration Curve of MEF (12.5-37.5µg/ml)

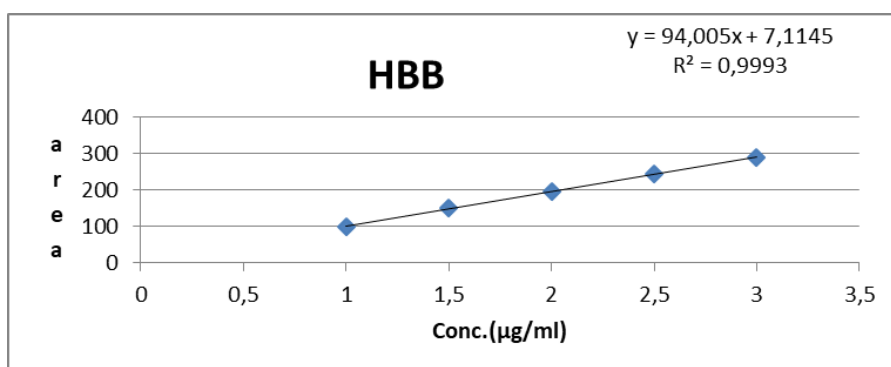


Figure 30. Calibration Curve of HBB (1-3µg/ml)

Table 3. Linearity data for MEF

Sr. No	Concentration ($\mu\text{g/ml}$)	Area	SD	%RSD
1	12.5	3405.647	0.517357	0.015191
2	18.75	5197.786	0.426888	0.008213
3	25	6712.509	0.32042	0.004773
4	31.25	8362.526	0.247275	0.002957
5	37.5	10164.48	0.381054	0.003749

Table 4. Linearity data HBB

Sr. No	Concentration ($\mu\text{g/ml}$)	Area	SD	%RSD
1	1	98.7486	0.405974	0.411119
2	1.5	150.6844	0.26081	0.173084
3	2	195.8558	0.306005	0.15624
4	2.5	242.4598	0.309686	0.127727
5	3	287.8732	0.313083	0.108757

Table 5. Precision and LOD, LOQ parameters for MEF and HBB

Parameters		MEF	HBB
Repeatability	n=6 %RSD	0.423	0.609
Interday	n=3 %RSD	0.832 - 0.940	0.554 - 0.740
Intraday	n=3 %RSD	0.249- 0.684	0.301- 0.350
LOD	n=6	1.068 $\mu\text{g/ml}$	0.077 $\mu\text{g/ml}$
LOQ	n=6	3.237 $\mu\text{g/ml}$	0.233 $\mu\text{g/ml}$

Table 6. Accuracy data of MEF and HBB

Drug	Level	Amount of sample taken ($\mu\text{g/ml}$)	Amount of standard spiked ($\mu\text{g/ml}$)	Total Conc. Found ($\mu\text{g/ml}$)	% Recovery \pm S.D. (n=3)
MEF	80 %	17	13.6	13.73	101.060 \pm 0.184
	100 %	17	17	17.12	99.857 \pm 0.805
	120 %	17	20.4	20.52	99.777 \pm 0.756
HBB	80 %	1.3	1.04	1.03	99.038 \pm 0.961
	100 %	1.3	1.3	1.29	100.025 \pm 0.770
	120 %	1.3	1.56	1.55	100.427 \pm 0.979

Table 7. Robustness data for MEF

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	7462.036	6105.909	6695.764	6810.021	7034.064	6499.808
2	7439.357	6107.162	6729.244	6789.608	7083.371	6449.785
3	7476.547	6160.882	6689.515	6689.515	7033.826	6474.611
% R.S.D	0.251	0.512	0.319	0.953	0.405	0.386

Table 8. Robustness data for HBB

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	216.121	193.900	197.208	203.712	188.220	216.121
2	215.148	194.853	196.609	205.143	188.033	215.148
3	216.526	193.785	195.224	203.697	186.330	216.526
% R.S.D	0.328	0.456	0.302	0.518	0.407	0.555

Table 9. Analysis of Marketed Formulation

Tablet	Label claim		Assay (% of label claim*) Mean \pm S. D.	
	MEF	HBB	% MEF	% HBB
Hyoscimax-MF	250mg	20mg	99.564 \pm 0.49	98.643 \pm 1.124

CONCLUSION

Forced degradation study of HBB and MEF was performed by RP-HPLC method in which Maximum degradation of MEF in base were found 18.33% for standard and 18.97 % for sample. And maximum degradation of HBB in thermal were found 20.06 % for standard and 20.78 for sample. The proposed sensitive RP-HPLC method gives accurate and precise results for determination of MEF and HBB in marketed formulation (tablet) without prior separation and is easily applied for routine analysis. The most striking feature of the method is its simplicity and rapidity. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision, LOD, LOQ and robustness. The proposed method was successfully applied to determination of these drugs in commercial tablets.

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