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Kinetics Study of Moprolol Degradation under Various Stress Conditions Using Stability Indicating Assay by RP-HPLC Method

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ABSTRACT

This study describes the development and validation of stability indicating HPLC method for moprolol, an antihypertensive drug. The drug was subjected to stress degradation under different conditions recommended by International Conference on Harmonization. The sample so generated was used to develop a stability-indicating high performance liquid chromatographic method for moprolol. The peak for was well resolved from peaks of degradation products, phenomenex C18 column (250 mm ×4.6 mm, 5 µ) at 37^0C, using an isocratic mobile phase consisting of mixture of 10 mM potassium dihydrogen phosphate buffer with 0.1% V/V TEA in Milli-Q water (pH 3, adjusted with OPA): ACN: Methanol (50:40:10 %V/V/V) at a flow rate of 1 mL $minute^{-1}$. The retention times were found to be 3.3 min, respectively. Detection was carried out using photodiode array detector. A linear response ($r > 0.99$) was observed in the range of 0-40µg/mL. The method was found to be specific and stability-indicating as no interfering peaks of degradants and excipients were observed. The method showed good recoveries (average 99.5%) and relative standard deviation for intra and inter-day were ≤ 2 %. The method was validated for specificity and robustness also.The proposed method is hence suitable for application of degradation kinetics of moprolol under different stress conditions employed. Degradation followed a pseudo-first-order kinetics, and rate constant (k), time left for 50% potency ($t_{1/2}$) and time left for 90% potency (t_{90}) were calculated. This method can also be applied in quality-control laboratories for quantitative analysis of both the drugs individually and in combination, since it is simple and rapid with good accuracy and precision.

Keywords: Moprolol, reversed-phase HPLC, stability-indicating assay, forced degradation studies, method validation, kinetics

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INTRODUCTION

Chemically moprolol (MOP) is 1-(2 methoxy phenoxy)-3-[(1-methyl ethyl) amino]-2-propanol; 1-(iso-propylamino)-3-(O-methoxy phenoxy)-2-propanol [1] (Figure 1). It is a Cardio selective ß$_1$-adrenergic blocker. It is used in the treatment of angina pectoris, glaucoma and hypertension [2].It is not official in any pharmacopoeia. A Survey of literature revealed few analytical methods for its estimation. MOP has been estimated in plasma by HPLC–GC following ophthalmic administration [3-5].However determination of drug in pharmaceutical dosage form has been reported by methods that includes spectrophotometric and chromatographic studies for its estimation have been reported till date [6-9]. No method for the analysis of MOP in presence of its degradation products is yet reported.

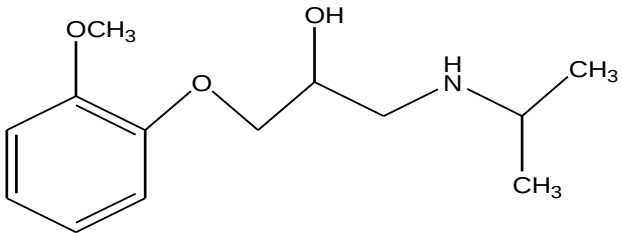

Figure 1: Chemical structure of MOP

The international Conferences on Harmonization (ICH) guideline[10, 11], entitled stability testing of novel drug substances and products needs that stress testing be carried out to illuminate the inherent stability characteristics of the active substance. The aim of this work was to develop stability indicating method for determination of MOP in presence of its degradation products. In the current study, stress degradation of MOP was caused by hydrolysis under acidic, basic, neutral conditions, oxidation with H_2O_2, dry heat degradation and photo degradation [12, 13]. The developed RP-HPLC method was validated following the ICH guidelines[14, 15] .However, kinetics studies and accelerated stability studies are important to rectify problems faced in quality control and to predict shelf life of pharmaceutical products. The novelty of the present work is that the recommended method indicates the first kinetics study of MOP degradation using RP-HPLC.

Hence it was thought worthwhile to develop spectrophotometric method for the same. In the present study we report two simple and sensitive spectrophotometric methods for the analysis of moprolol from bulk and pharmaceutical dosage forms.

MATERIALS AND METHOD

Chemicals and Reagents:

Authentic sample of MOP (99.1 % as on dried basis) was kindly gifted by strides and chemical specialty Ltd Mangalore. As this drug has no marketed formulations yet, we have formulated

mouth dissolving tablets (Tablet1 and Tablet 2) by varying the ratio of using most commonly used excipients like starch, MCC, talc and magnesium stearate by keeping the strength as constant (25 mg of moprolol) and analyzed the drug HPLC grade methanol and acetonitrile were purchased from Merck chemicals, Mumbai. The water for HPLC was prepared by double glass distillation and filtered through Milli Q water purification system. Potassium dihydrogen phosphate and orthophosphoric acid (OPA) were obtained from SD fine chemical Ltd. Mumbai, India.

HPLC Instrumentation:

HPLC consists of Shimadzu LC-10 ADVP isocratic solvent delivery system, SIL- 10ADVP auto-injector, CTO-10ASVP column oven, SPD-10A UV/Vis detector, SCL-10 AVP System controller, a rheodyne manual injector model 7725i with 20µL loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system. All data integration was performed using class VP software.

Mobile Phase Preparation:

The mobile phase consisted of a mixture of 10 mM potassium dihydrogen phosphate buffer with 0.1% V/V TEA in Milli-Q water (pH 3, adjusted with OPA): Acetonitrile: Methanol (50:40:10 %V/V/V). The mobile phase was filtered through Millipore filter (0.45 µm) and was degassed prior to use and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai).

Diluent Preparation:

Buffer: Acetonitrile: Methanol (50:40:10 %V/V/V) used as a diluent.

Standard Preparation:

The assay concentration of MOP was selected as 10 µg mL^{-1}. It was prepared by weighing 100 mg of MOP and it was dissolved in 100 mL methanol to get concentration of 1 mg mL^{-1} of MOP. It was diluted with mobile phase to get a concentration of 100 µg mL^{-1} (working stock). Further dilutions were carried out using the mobile phase to get resultant concentration of 10 µg mL^{-1}.

Test Preparation:

Twenty tablets were weighed and the average weight of tablet was determined (equivalent to 25mg each of MOP) were ground to powder. From these, five tablets were weighed and transfer into a 500 ml volumetric flask. About 50 mL methanol was added and sonicated for a minimum 30 minute with intermittent shaking. Then content was brought back to room temperature and diluted to volume with methanol. The sample was filtered through 0.45µm nylon syringe filter. Take 10 mL this filtrate solution in 50 mL volumetric flask and make up to mark mobile phase as a diluent. The concentration obtained was 10 µg $mL^{-1}$of MOP.

Chromatographic conditions:

Chromatographic analysis was performed on an analytical column used was Phenomenex C18 column (250 mm ×4.6 mm, 5 µ particle size). All analysis was carried out at a temperature of 25°C under isocratic conditions. The mobile phase consists of 10 mM potassium dihydrogen phosphate buffer with 0.1% V/V TEA in Milli-Q water (pH 3, adjusted with OPA): acetonitrile: methanol (50:40:10 %V/V/V). The flow rate was 1 mL min^{-1}, the volume of injection was 20 µL, total run time was 10 minutes, and the detection was made at 254 nm and the injection volume was 20 µL.

Forced degradation studies:

Forced degradation studies under acidic, alkaline and neutral conditions were performed by refluxing using a heating mantle with temperature control (Tempad, Mumbai, India). The photo stability studies were carried out in a stability chamber (KBF 240, WTB Binder, Tuttlingen, Germany) equipped with light sources as defined under option 2 of the ICH guideline Q1B. The light bank consisted of a combination of two black light Osram L73 lamps and four Osram L20 lamps. The black light lamp (L73) had a spectral distribution between 345 and 410 nm with maximum at 365 nm. The output of white fluorescent lamps (L20) was similar to that specified in ISO 10977 (1993). Both UV and visible lamps were switched on simultaneously. The chamber was maintained at 40° and 75% RH.

Acidic condition:

Neutral hydrolysis of the drug was carried on solution prepared by dissolving 25 mg of the drug in water. The samples were exposed to stress conditions viz. 12 h, 24h, and 48h days, reflux. Acid hydrolysis of the drug was carried out in presence of different concentrations of HCl (0.5N and 1N).The solution was refluxed in 1 N hydrochloric acid solution. Immediately after making up the volume, the solution was transferred to round bottom flask and kept for refluxing at 80ºC, 1 mL sample were taken at different time interval 0, 4, 8, 12, 24, 48, 72 and 96 h and the sample were neutralized with 1M sodium hydroxide solution. The standard solution of MOP in 10 mM potassium dihydrogen phosphate buffer with 0.1% V/V TEA in Milli-Q water (pH 3, adjusted with OPA): acetonitrile: methanol (50:40:10 %V/V) was prepared which was considered as 100% for degradation study. After exposure for the required duration of time the samples were diluted to a concentration of 10 µg mL^{-1} with the mobile phase. The samples were then injected into the HPLC system after filtration.

Alkaline condition:

While for alkaline hydrolysis 25 mg of the drug was dissolved in 0.1Nsodium hydroxide solution. Immediately after making up the volume, the solution was transferred to round bottom flask and kept for refluxing at 80ºC, 1 mL sample were taken at different time intervals 0, 4, 8, 12, 24, 48, 72

and 96 h and the sample were neutralized with 1 N hydrochloric acid solution. The standard solution of MOP in methanol was prepared which was considered as 100% for degradation study. After exposure for the required duration of time the samples were diluted to a concentration of 10 µg mL^{-1} with the mobile phase. The samples were then injected into the HPLC system after filtration.

Thermal condition:

Thermal degradation was performed by exposing solid drug at 80˚ C for 72h. Resultant chromatogram of thermal degradation study indicates that MOP is found to be stable under thermal degradation condition.

Oxidative stress:

The drug solution of 10 µg $mL^{-1}$was prepared in different concentrations of hydrogen peroxide solution (3, 10 and 0.3%). The studies were carried out in 3, 10, and, 0.3% H_2O_2 by studying 1 mL sample at different time interval 0, 4, 8, 12, 24, 48, 72 and 96 h. After exposure for the required duration of time the samples were injected into the HPLC system after filtration.

Photo degradation:

Some drug molecules undergo degradation upon exposure to light, which necessitate special storage conditions and protection. Sample solution of the drug 10 µg mL^{-1} concentration was prepared. The samples were then kept in stability chamber (KBWF 240, WTB Binder, Germany) equipped with light source (option 2) as specified in the ICH guidelines Q1B and maintained at 40º and 75% RH. The samples of both solution and powder were kept in dark for the same period. Samples were withdrawn at different time periods and analyzed after sufficient dilution.

Method validation:

Specificity:

The specificity of the method was established through determination of the drug in the presence of degradation products as well as the through determination of peak purity for the drug in the presence of degradation products using PDA detector.

Linearity:

From the working stock solution (100 µg mL^{-1}), the serial dilutions were done to get the concentration of 1, 5, 10, 15, 20, 25, 30, 35 and 40 µg mL^{-1}respectively. A volume of 20 µL from each concentration of the solution was injected and chromatograms were recorded under the optimized chromatographic conditions. The peak area of the drug was plotted against the concentration of the drug.

Repeatability:

Repeatability is the result of the method operating over a short time interval (within a day) under the same conditions. The peak area of assay concentration of the drug (10 µg mL^{-1}) was analysed six times on the same day. The percentage relative standard deviation (%RSD) was calculated for the resultant peak area.

Intermediate precision:

To assess the degree of reproducibility of the method, assay concentration (10 µg mL^{-1}) was analysed on different day. The assay procedure was repeated six times and the chromatogram was recorded and the %RSD was calculated.

Accuracy:

Accuracy of the method was demonstrated by using drug substance spiked at three different concentration levels in triplicate. The analyses were carried out at 25%, 50% and 75% of specification limit as per ICH guide lines. The mean recoveries of all the impurities were calculated.

Robustness:

Robustness was done by small deliberate variations in method parameters like Influence of variations in flow rate (±0.1mL min^{-1}), Influence of variations of pH of mobile phase (± 0.2), Influence of change in temperature (± 5˚C) Influence of variations in composition of mobile phase (± 2 %).

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

LOD and LOQ were calculated based on signal to noise ratio and illustrated in results.

System suitability parameters:

These parameters include plate number (**N**), capacity factor (**k'**), asymmetry factor, and relative standard deviation (RSD) for peak area for repetitive injections. These parameters were within the acceptable limits.

Stock solution stability:

The freshly prepared standard stock solution of MOP 10 µg $mL^{-1}$was stored in the refrigerated temperature[16]. The stability of the stock was analysed after one month duration and compared with the initial concentration.

RESULTS AND DISCUSSION

Development and Optimization of the HPLC Method:

Proper selection of the methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and solubility. MOP is dissolved in polar solvent hence RP-HPLC was selected to estimate them. To develop a rugged and suitable HPLC method for the

quantitative determination of MOP, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other chromatographic conditions. Our preliminary trials using different composition of mobile phases consisting of water with methanol or acetonitrile, did not give good peak shape. By using 10 mM potassium dihydrogen phosphate buffer with 0.1% V/V TEA in Milli-Q water (pH 3, adjusted with OPA) and keeping mobile phase composition as phosphate buffer : acetonitrile: methanol (50:40:10 %V/V/V), best peak shape was obtained. For the selection of organic constituent of mobile phase, methanol was chosen to reduce the longer retention time and to attain good peak shape. (Figure 2 and Figure 3) represent the chromatograms of standard and test preparation respectively.

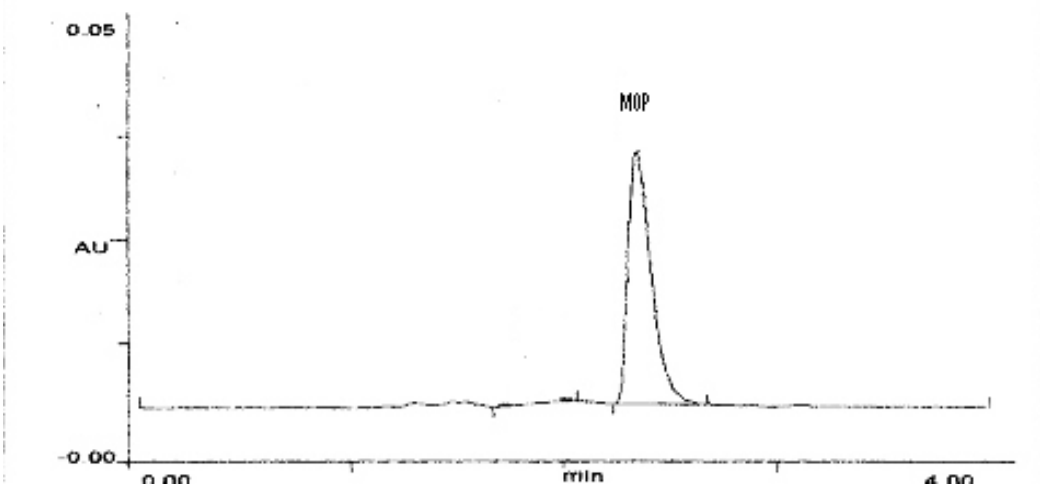

Figure 2: Chromatogram of standard preparation

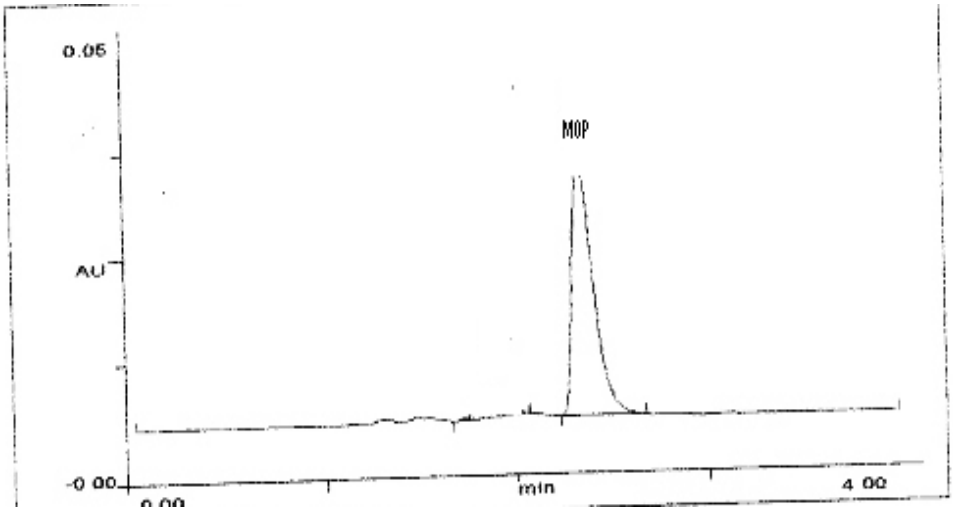

Figure 3: Chromatogram of test preparation

Forced Degradation Study:

During the study it was observed that upon treatment of MOP with different strengths of acid (0.5 N, 1 N HCl), base (0.1 N, 0.5 N NaOH) and hydrogen peroxide (10%, 3%, 0.3%).The degradation

was observed only with the higher strengths of alkali (0.1 N NaOH), acid (1 N HCl) and hydrogen peroxide (3 %, 10%) whereas with the lower strengths of alkali (0.5N NaOH), acid (0.5 N HCl) and hydrogen peroxide (0.3%) no degradation was observed. (Table 1) indicates the extent of degradation of MOP under various stress conditions.

Acid induced-degradation:

The drug substance showed sufficient degradation while refluxing for 4h and 6h at 80°C in 1N HCl. The single degradation product formed with retention time of 3.87, 4.67 and 5.75 minutes. No further significant increase in % degradation was observed till 12 h. The assay of the active substance in acid degraded sample is shown in (Figure 4, Table 2).

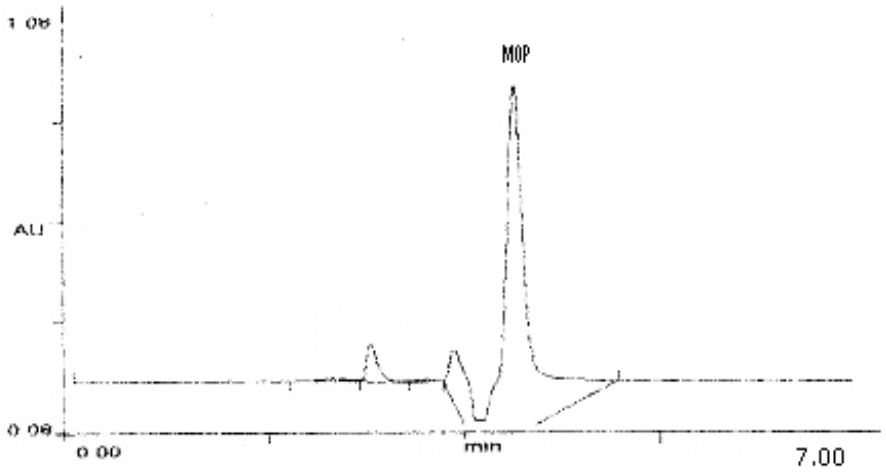

Figure 4: Chromatogram of acidic forced degradation study

Alkali induced-degradation:

The chromatogram of the base induced degraded samples of MOP showed additional peaks at 1.6, 1.9, 2.5 and 3.2 minutes of retention time. As compared to acidic conditions, MOP as found to be more susceptible to alkaline conditions. Refluxing at 80°C for 12 h around 10% of the degradation was recorded. The assay of the active substance in base catalyzed degraded sample was found in (Figure 5) and results shown in (Table 1).

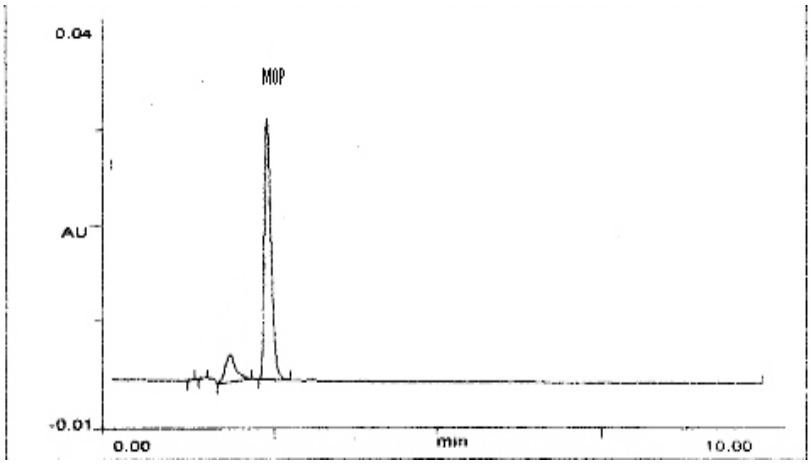

Figure 5: Chromatogram of alkali forced degradation study

Oxidative degradation:

Drug substance was found to be highly susceptible to oxidation and most of the drug decomposed within 1.5 h. The drugs showed sufficient degradation when refluxed with 3% H_2O_2 for 1 h and 10% H_2O_2 for 0.5 h respectively. The degradation products appeared at 2.26, 2.72 and 3.43 minutes. (Figure 6, Table 1)

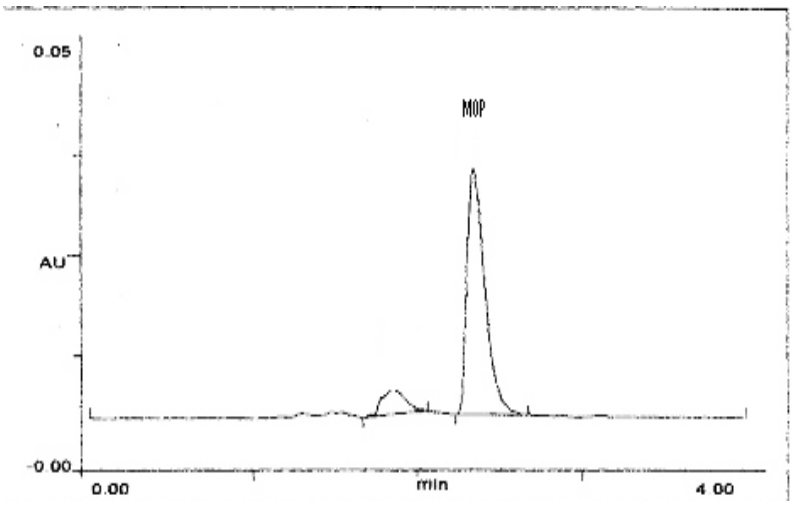

Figure 6: Chromatogram of oxidative forced degradation study

Photolytic degradation:

Drug substance was exposed to photolytic degradation at 1.2 million lux h for a period of 48 h and 96 h. No degradation peak was observed at 48 h time interval while additional degradation peaks were observed at 96 h. Retention time of those peaks are shown in the chromatogram. The assay of the active substance in photo degraded sample was shown in (Figure 7, Table 1)

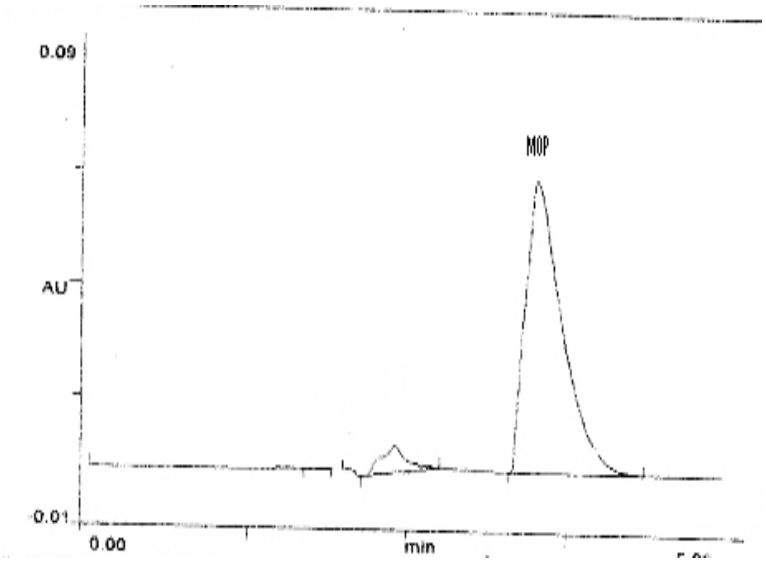

Figure 7: Chromatogram of UV-light degradation study

Table 1: Degradation of MOP under various stress conditions

Condition		% Drug Area	%Total Degradation
Acid Degradation	1 N HCl, 4 Hour	99.7	0.3
	1 N HCl, 6 Hour	96.81	3.82
Alkali Degradation	0.1 N NaOH, 2 Hour	87.12	12.88
	0.1 N NaOH, 4 Hour	89.10	10.9
Peroxide Degradation	3 % H_2O_2 , 0.5 Hour	99.51	0.49
	3 % H_2O_2 , 1 Hour	75.98	24.02
	10 % H_2O_2 , 0.5 Hour	74.07	25.93
Photolytic Degradation	48 Hour	No Degradation	
	96 Hour	93.00	7.0
Thermal Degradation	100 º C	No Degradation	
	120 º C		

Thermal degradation:

Peak purity studies on the drug peak showed purity angle (PA) value of 0.049 and purity threshold (TH) value of 0.259. As the purity angle value was found to be less than purity threshold the method was found to be specific to the drug. Studies performed on accelerated stability samples also showed good separation of drug peak from other peaks. This indicated that the method was specific to the drug shown in (Figure 8)

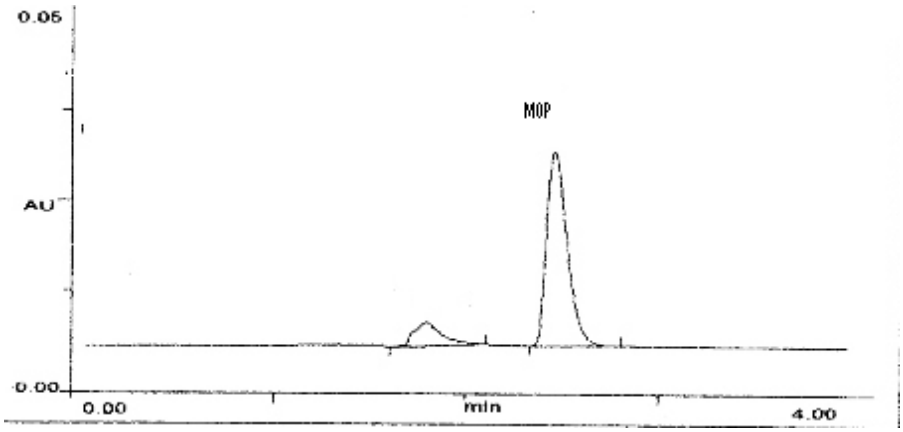

Figure 8: Chromatogram of thermal degradation study

Method Validation:

Specificity:

The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method was eluted by checking the peak purity of MOP during the force degradation study. The peak purity of the MOP was found satisfactory (0.9999) under different stress condition. There was no interference of any peak of degradation product with drug peak shown in (Figure 9-10)

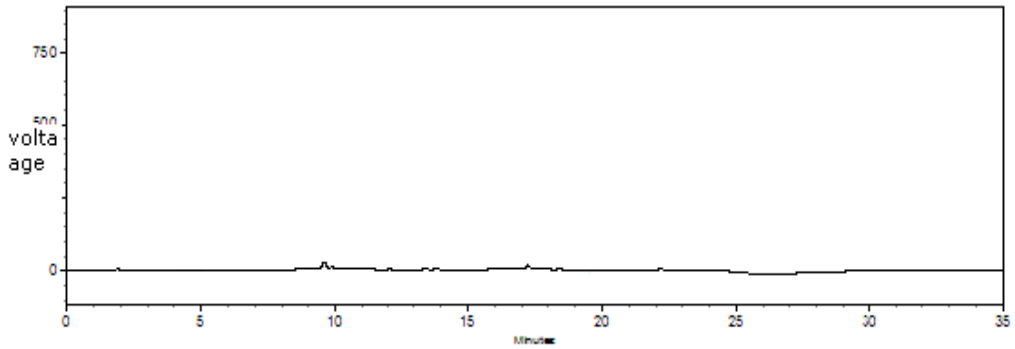

Figure 9: Chromatogram of blank preparation

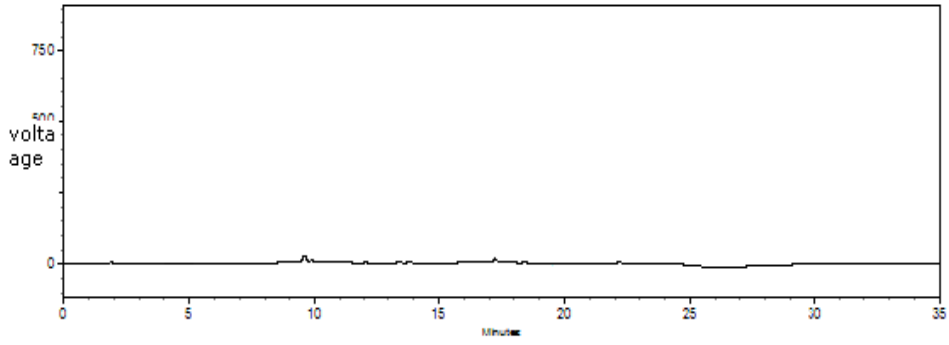

Figure 10: Chromatogram of placebo preparation

Linearity:

Calibration curve were achieved in a concentration range from 0-40 µg mL^{-1} for MOP. The response of the drug was found to be linear in the examination concentration range with correlation coefficient 0.999891 (Figure 11).

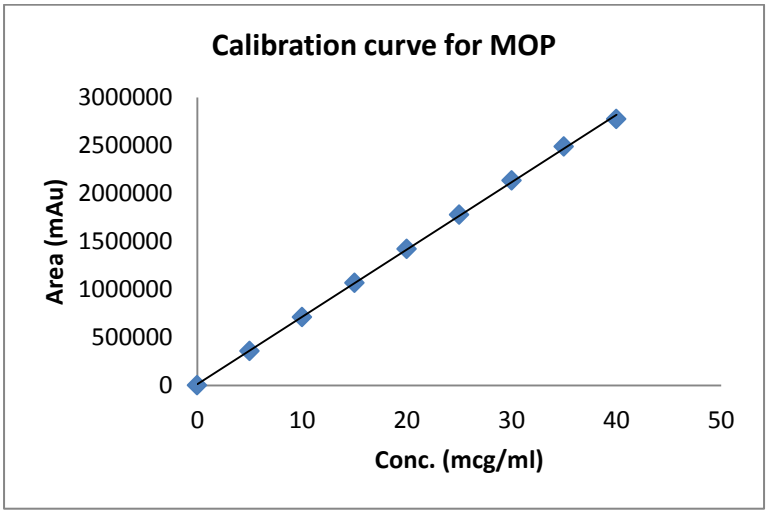

Figure 11: Linearity curve for MOP

The result of repeatability and intermediate precision study are shown in (Table 2).The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were < 0.65 % and < 0.71 to 0.23 %, respectively, which confirm that method was precise.

Table 2: Evaluation data of precision study

	Set No.		MOP			
			% Assay	%Assay Mean	%RSD	Acceptance Criteria
Method precision (n=6)	1		99.21	100.28	0.65	% RSD < 2
	2		101.12			
	3		99.99			
	4		98.76			
	5		101.59			
	6		101.05			
Intermediate precision (n= 3)	Day-1	1	99.98	99.94	0.23	
		2	99.97			
		3	99.87			
	Day-8	1	99.89	99.59	0.71	
		2	98.98			
		3	99.91			

Accuracy:

The HPLC area responses for accuracy determination are depicted in Table 3. The result shown that best recovery (98.66-100.61 %) of the spiked drug was obtained at each added concentration,

indicating that the method was accurate. Chromatogram obtain during accuracy study were shown in (Figure 12-14).

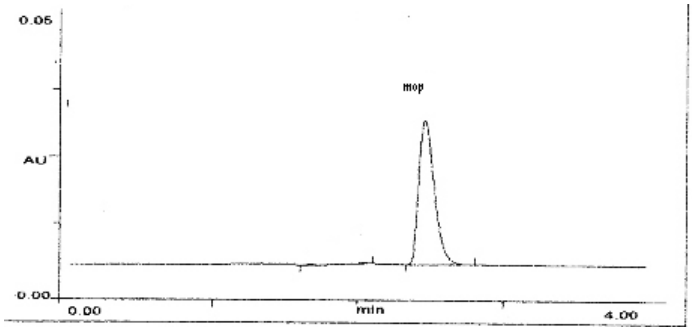

Figure 12: Accuracy level study chromatogram of level 1(25%)

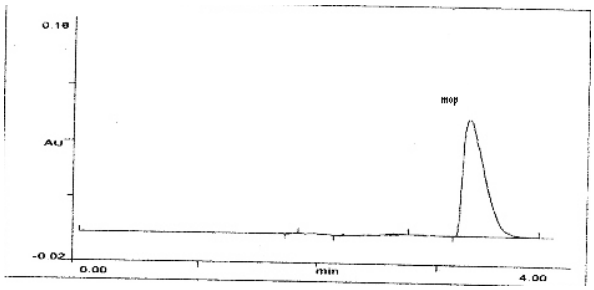

Figure 13: Accuracy level study chromatogram of level 2(50%)

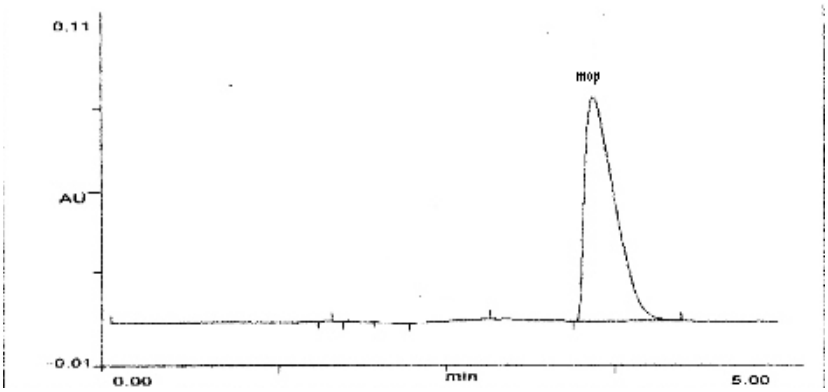

Figure 14: Accuracy level study chromatogram of level 3(75%)

Table 3: Evaluation data of accuracy study

S.No.	Concentration used for estimation (%)	Amount Present (µg mL^{-1})	Amount Added (µgmL^{-1})	Amount Estimated* (µg mL^{-1})	Amount Recovered* (µg mL^{-1})	% Recovery	S.D.	%RSD
1.	25	15.1	3.75	18.84	3.74	99.73		
2.	25	15.1	3.75	18.80	3.70	98.66		
3.	50	15.1	7.55	22.56	7.46	98.80	0.972	0.9818
4.	50	15.1	7.55	22.57	7.47	98.94		
5.	75	15.1	11.33	26.43	11.33	100.00		
6.	75	15.1	11.33	26.50	11.40	100.61		

[a] Each value corresponds to the mean of three determinations

Robustness:

The method was demonstrated to be robust over an acceptable working range of its HPLC operational conditions. The system suitability results within the acceptable limits and selectivity of individual substances were also not affected when subjected deliberately for varied chromatographic conditions. The result of the study confirms the robustness of the method. The result of robustness study of the developed assay method was established in (Table 4) and shown in (Figure 15-21)

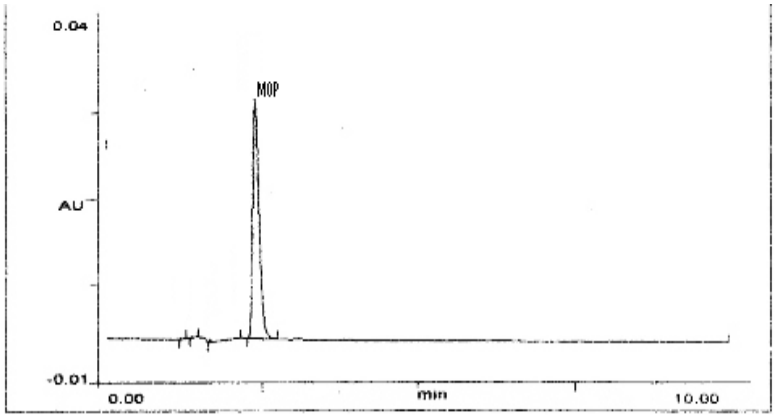

Figure 15: Standard chromatogram (0.5 mL min^{-1} flow rate)

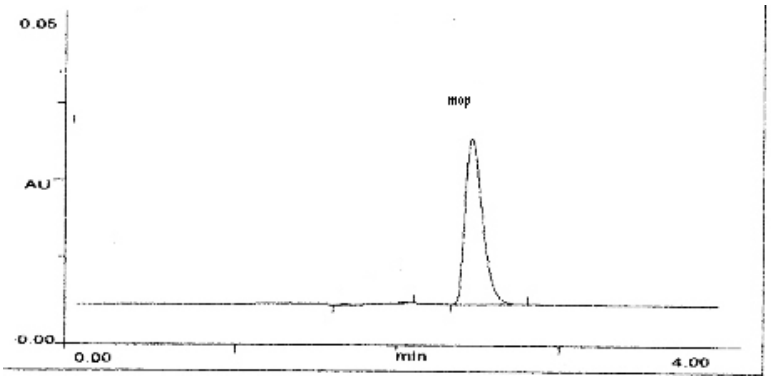

Figure 16: Standard chromatogram (1.0 mL min^{-1} flow rate)

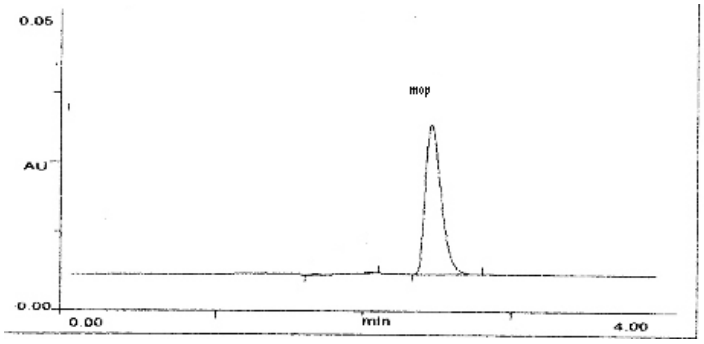

Figure 17: Standard chromatogram (Buffer: Acetonitrile: Methanol 50:40:10%V/V/V)

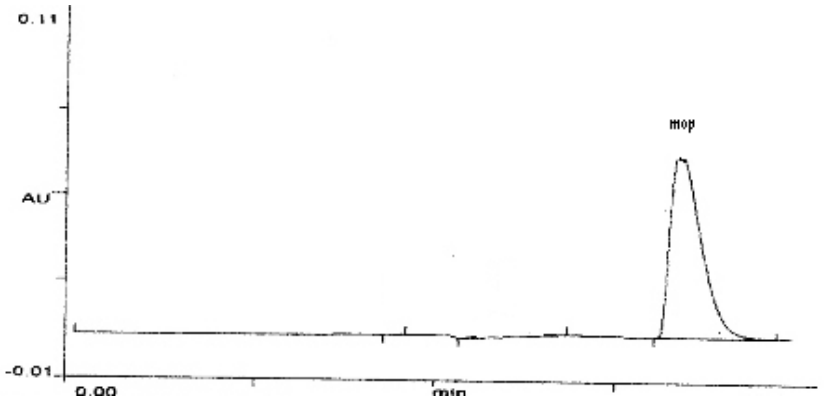

Figure 18: Standard chromatogram (Buffer: Acetonitrile: Methanol 65:25:10%V/V/V)

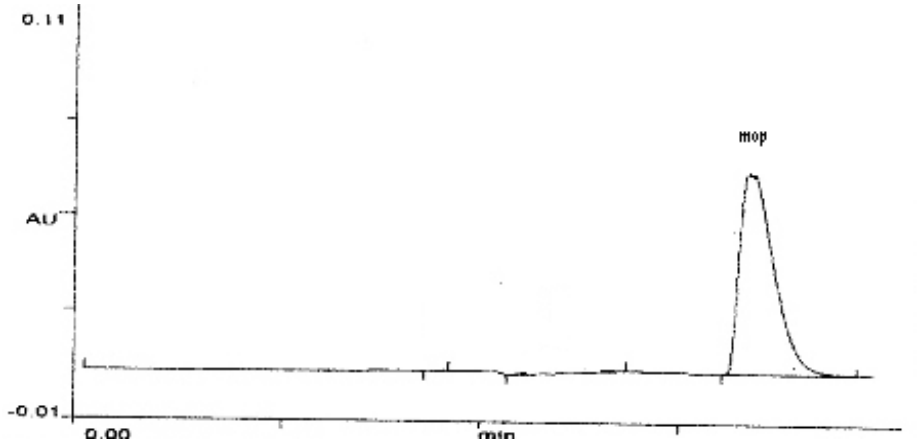

Figure 19: Standard chromatogram (pH 3)

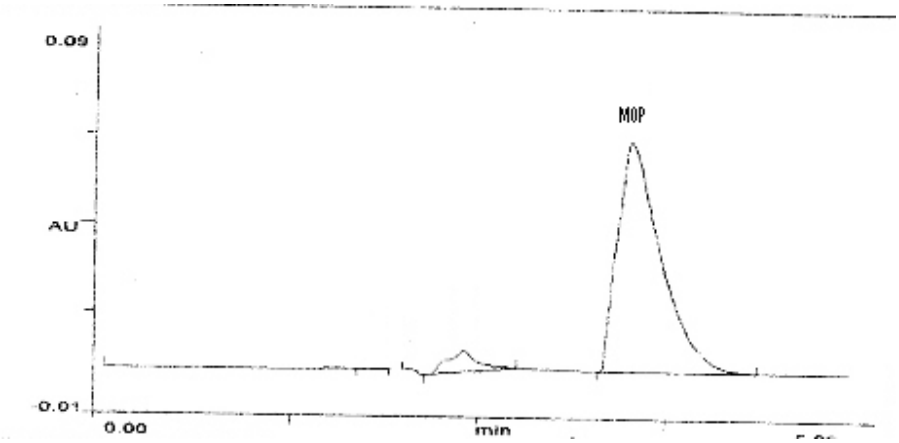

Figure 20: Standard chromatogram (pH 3.5)

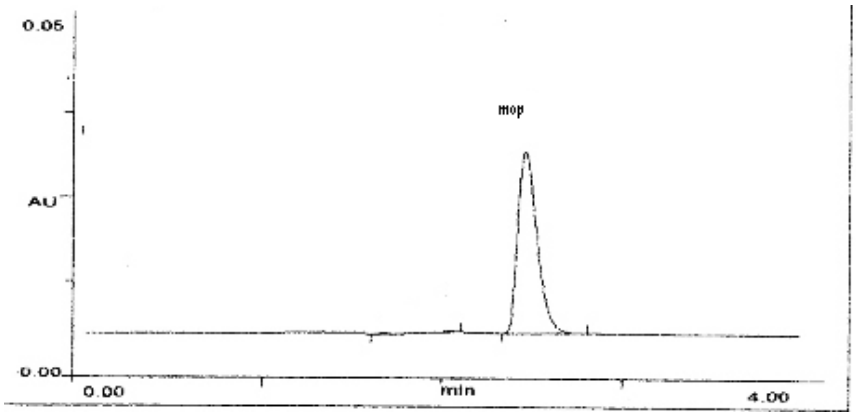

Figure 21: Standard chromatogram (column change)

Table 4: Evaluation data of robustness study

Intervals	% Assay for test solution stored at 2 -5˚ C	% Assay for test solution stored at ambient temperature
Initial h	100.1	101.5
12h	99.9	99.8
24h	99.8	99.1
36h	101.6	99.9
48h	99.3	99.6

Stability of the solution:

The test solution is stable at least for 48h at 25 ^{0}C and at 5 ^{0}C. (Table 5) shows the results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 h at 2 - 5˚ C and at ambient temperature with the consideration of< 2.0 % in % assay value difference against interval value.

Table 5: Evaluation data of solution stability study

S.No	Nature and composition of Mobile phase(%V/V/V)	Flow rate (mL min^{-1})	P^{H}	Rt (minutes)	Tailing factor	Asymmetric Factor	Capacity Factor	No. of Theoretical plates
1.	ACN: buffer: methanol (65:25:10)	0.8	2.8	3.52	2.02	1.96	0.99	733
2.	Buffer: ACN: Methanol (50:40:10)	1.0	3.0	3.33	1.67	1.81	1.15	3443

System suitability:

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate, resolution and % RSD of peak area were determined for same. Acceptance criteria for system suitability, asymmetry not more than 2.0, theoretical plate not less then 3000 for moprolol and %

RSD of peak area not more then 2.0, were full fill during all validation parameter which is shown in (Table 6).

Table 6: System suitability parameters of MOP

S.no.	Parameters	MOP
1.	Tailing factor	1.67
2.	Asymmetrical factor	1.81
3.	Theoretical plates	3443
4.	Capacity factor	1.15
5.	Retention Time (min)	3.3

Kinetics investigation:

Treatment of MOP under specified stress conditions resulted in a gradual decomposition of MOP in acidic, alkali and oxidative conditions. Since plots of log concentration versus time resulted in straight lines, the degradation of MOP followed first-order kinetics [17] as a linear relationship between log percentage of MOP remaining and time was established, having good correlation coefficients (Fig. 22). The kinetics parameters are presented in (Table VII)**.** Rate constant(K),time left for 50% potency ($t_{1/2}$) and time left for 90% potency (t_{90}) for each stress condition were calculated using Eqs.(1),(2) and (3),respectively.

$$\log [C_1]=\log[C_0]-K_t/2.303 \quad (1)$$

$$t_{1/2}=0.693/K \quad (2)$$

$$t_{90}=0.105/K \quad (3)$$

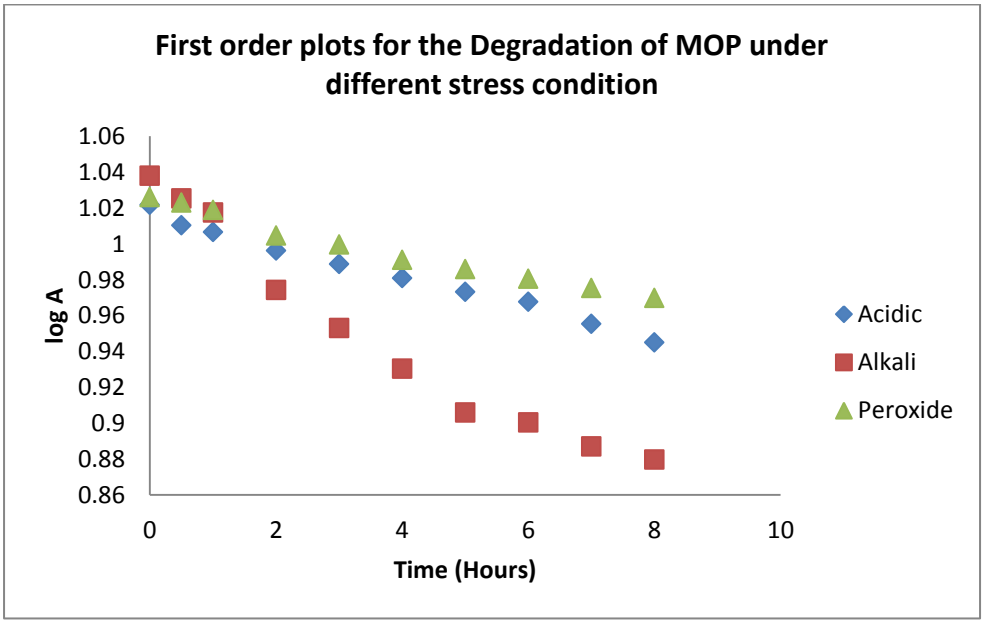

Figure.22: First order plots for the degrdartion of MOP under different stress conditions.

Table 7: Summary of MOP degradation kinetics

Stress condition	*K*(1/day)[a]	$t_{1/2}$(days)[b]	t_{90}(days)[c]
Acid	$1.78X10^{-1}$	3.89	0.59
Alkali	$3.82\ X10^{-1}$	1.81	0.28
Peroxide	$2.00\ X10^{-1}$	3.47	0.525

[a]Rate constant per day

[b]Half life

[c]Time left for 90% potency

Where K is the rate constant, $[C_0]$ is the concentration of MOP at time t=0 and $[C_t]$ is its concentration at time t. The K values per day were found to be $3.82\ X10^{-1}$, $1.78X10^{-1,}$ and $2.00\ X10^{-1}$ for 0.1N NaOH, 1N HCl and 3%H_2O_2 respectively. Extensive degradation was observed in oxidative stress conditions, where K value was found to be the highest among all the tested conditions; $t_{1/2}$ and t_{90} were found to be lowest (1.81 and 0.28 days) for basic condition (0.1N NaOH) and highest (3.89 and 0.59 days) for acidic(1N HCl)condition.

CONCLUSION

Thus the study shows that MOP undergoes degradation in acidic, alkaline and oxidative conditions whereas it is relatively stable when exposed to dry heat and photolytic conditions. A stability-indicating method was developed, which resolved all the degradation products formed under variety of conditions. The degradation products formed under acid, alkali and oxidative condition are same which is confirmed from UV spectrum of the degraded product. The degradation of MOP was found to follow a first-order reaction. The drug was extensively degraded under oxidative stress conditions. The $t_{1/2}$ values under different stress conditions were found to be in the following order: 1N HCl >3%H2O2> >0.1N NaOH. The method proved to be simple, accurate, precise, specific and selective. Hence it may be used to assay of the product during stability studies.

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