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Stability indicating reverse phase LC method development and validation for simultaneous estimation of metoprolol succinate and chlorthalidone in combined tablet dosage form

Mohammad Yunoos*[1] and D. Gowri Sankar[2]

[1]*Department of Pharmaceutical Analysis, Bapatla College of Pharmacy, Bapatla, Guntur (Dist.), Andhra Pradesh, India*
[2]*College of Pharmaceutical Sciences, Andhra University, Visakhapatnam*

ABSTRACT

A simple and precise stability indicating RP-HPLC method was developed and validated for simultaneous determination of Metoprolol succinate and Chlorthalidone in bulk and combined tablet formulation. Chromatography was carried out on Inertsil ODS C_{18} (250 x 4.6 mm, 5µ particle size) column in an isocratic mode with mobile phase containing phosphate buffer (adjusted to pH 5.5 with dilute othophosphoric acid and acetonitrile in the ratio of 55:45% v/v at a flow rate of 0.8 ml/min. The analyte was monitored using PDA detector at 219 nm. The retention time was found to be 3.763 min and 4.924 min for Metoprolol succinate and Chlorthalidone respectively. The proposed RP-HPLC method was found to be having linearity in the concentration range of 12.5-75 µg/ml for Metoprolol succinate and 3.125-18.75 µg/ml for Chlorthalidone with correlation coefficient value of 0.999 respectively. The mean % recoveries obtained were found to be 99.32-99.98 % for Metoprolol succinate and 99.52-99.84 % for Chlorthalidone respectively. Stress testing which covered acid, base, peroxide, UV light, neutral and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to ICH guide lines. Thus the proposed method can be successfully applied for the stability indicating simultaneous determination of Metoprolol succinate and Chlorthalidone in bulk and combined tablet dosage form and in routine quality control analysis.

Keywords: Metoprolol succinate, Chlorthalidone, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

Metoprolol succinate

Chemically (Fig.1), it is {butanedioic acid; 1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-ylamino) propan-2-ol. It has a molecular formula of $C_{34}H_{56}N_2O_{10}$ and molecular weight of 652.8 g/mol. Metoprolol succinate is an antihypertensive agent (β_1-Adrenergic blocker). Adrenergic beta-antagonists are used for treatment of hypertension, cardiac arrhythmias, angina pectoris, glaucoma, migraine headaches and anxiety. Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at β_1-adrenergic receptors in the heart. β_1-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

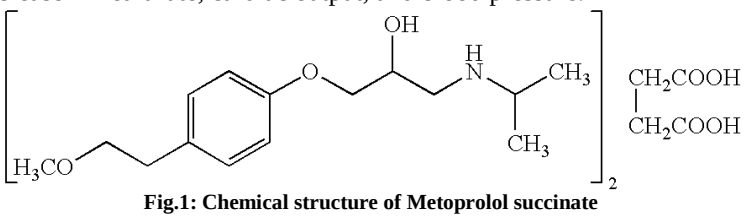

Fig.1: Chemical structure of Metoprolol succinate

Chlorthalidone

Chemically (Fig.1), it is 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide. It has a molecular formula of $C_{14}H_{11}ClN_2O_4S$ and molecular weight of 338.766 g/mol. It is used as an antihypertensive agent, diuretic and sodium chloride symporter inhibitor. Chlorthalidone inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, Chlorthalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism.

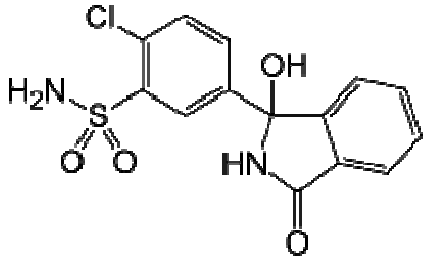

Fig.2: Chemical structure of Chlorthalidone

Literature survey revealed that few analytical methods were reported so far for both drugs in combination or in alone like RP-HPLC method in biological fluids [1], RP-HPLC [2-5], HPTLC [6] and Spectrophotometric methods [7-12] in pharmaceutical dosage forms. However there was no stability indicating method reported for this drug combination and hence the present study was aimed to develop a simple, fast, economical, selective, accurate, precise and sensitive stability indicating RP-HPLC method for the simultaneous determination of Metoprolol succinate and Chlorthalidone in bulk and combined tablet dosage forms, suitable for routine quality control analysis.

MATERIALS AND METHODS

Chemicals

The Pharmaceutical grade pure samples of Metoprolol succinate and Chlorthalidone were received as gift samples from Sun Pharmaceutical Industries Ltd., Mumbai. HPLC grade water, methanol and acetonitrile were purchased from E.Merck. Chem.ltd., Mumbai. All the chemicals used were of analytical reagent grade (E.Merck). Fixed dose combination tablet formulations (Vinicor-D) containing 25 mg of Metoprolol and 6.25 mg of Chlorthalidone (Manufactured by IPCA Laboratories Ltd., Mumbai) were procured from local market.

Instrumentation

Quantitative HPLC was performed on Waters 2695 separation module Alliance Isocratic HPLC system and PDA Detector 2996 series equipped with auto injector using empower software. An UV-3000+ series UV/Visible double beam spectrophotometer from LABINDIA with 1 cm matched quartz cells was used for all spectral measurements using UV win 5 software.

Chromatographic conditions

Mobile phase composition	Phosphate buffer (adjusted to pH 5.5 with dilute OPA): acetonitrile in the ratio of 55:45 %v/v
Stationary phase	Inertsil ODS C_{18} column (250 x 4.6mm, particle size 5µ)
Detector wave length	219 nm
Run time	10 min
Flow rate	0.8 ml/min
Injection volume	20µl
Colum temperature	30^0C (ambient)

Preparation of Phosphate buffer

Accurately weighed quantity of 1.36 gm of Potassium dihydrogen orthophosphate was transferred into a 1000ml volumetric flask. About 900ml of HPLC grade water was added and degassed by subjecting to sonication for 5 min and final volume was made up to the mark with water. Filtered through 0.45µ membrane filter using vacuum filtration and then P^H of the solution was adjusted to 5.5 with dilute orthophosphoric acid solution.

Preparation of Mobile phase

Phosphate buffer and acetonitrile were mixed in the ratio of 55:45 %v/v and then degassed by subjecting to sonication for 10 min and resultant solution used as mobile phase after filtration through 0.45µ membrane filter using vacuum filtration assembly.

Preparation of diluent
Mobile phase was used as diluent.

Preparation of standard solution
Accurately weighed and transferred 25 mg of Metoprolol and 6.25 mg of Chlorthalidone reference standards into a 50 ml clean dry volumetric flasks separately, 30 ml of diluent was added, sonicated to dissolve for 5 minutes and then made up to the final volume with diluent to obtain stock solution of concentration of 500µg/ml of Metoprolol and 125µg/ml of Chlorthalidone respectively. From the above stock solution, 1 ml each was pipette out separately in to a 10 ml volumetric flasks and then volume was made up to mark with diluent to obtain 50µg/ml of Metoprolol and 12.5µg/ml of Chlorthalidone working standard solutions respectively.

Preparation of Sample solution
20 tablets were accurately weighed and determined average weight of the tablets. An amount of powder equivalent to 25mg of Metoprolol and 6.25 mg of Chlorthalidone were weighed accurately and transferred into a 50ml volumetric flask, 30ml of diluent was added, sonicated for 10 min and volume was made up with diluent. Filtered through 0.45µ membrane filter. From the filtered solution, 1ml was pipette out into a 10 ml volumetric flask and then volume was made up to mark with diluent to obtain final concentration of 50µg/ml solution of Metoprolol and 12.5µg/ml solution of Chlorthalidone respectively. Then Injected 20µl of filtered portion of the blank, sample and standard preparation into the chromatograph separately. Recorded the responses for the major peaks. Calculated the content of Metoprolol and Chlorthalidone present in each tablet.

Method validation
Analytical validation parameters for this proposed method were determined according to ICH guidelines.

System suitability
System suitability test should be carried out to verify that the analytical system is working properly and can give accurate and precise results. Standard solutions were prepared as per the test method and injected five times into the chromatographic system. The system suitability parameters were evaluated for tailing factor, retention times and theoretical plates of standard chromatograms. The results for system suitability studies are presented.

Specificity
Specificity is the ability to assess unequivocally the analyte in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the chromatograms obtained from the drug standards with that of obtained from the tablet preparations. The retention times of the drug standards and the drug from sample preparations were same, so the method was specific without interference from excipients in the tablets.

Linearity
The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different concentration levels (25-150%) of linearity solutions were prepared by diluting aliquots (0.25- 1.50 ml) of standard stock solution (500µg/ml of Metoprolol and 125µg/ml of Chlorthalidone) in to each 10 ml volumetric flasks (6 no's) and diluted to final volume with diluent to obtained concentrations in the range of 12.5-75 µg/ml for Metoprolol and 3.125-18.75 µg/ml for Chlorthalidone respectively to demonstrate linearity for assay. Then injected 20µl solution of each concentration into the chromatographic system and the chromatograms were recorded. The calibration graphs were plotted between amount of drug concentration (µg/ml) and chromatographic peak areas (AU) of Metoprolol succinate and Chlorthalidone respectively. The linearity of the proposed method was then evaluated by linear regression analysis. The correlation coefficient, slope and intercept were calculated from the graph and are reported.

Precision
System precision (Repeatability)
System precision was carried out by six replicate injections of the working standard solution at 100% concentration level into the chromatographic system. The corresponding peak areas of Metoprolol succinate and Chlorthalidone were measured and % RSD was calculated.

Method precision
The method precision study was performed by injecting six sample preparations of marketed formulations into the chromatographic system. The corresponding peak areas of Metoprolol succinate and Chlorthalidone were measured and % RSD was calculated.

Inter-day precision
Inter-day precision was performed by injecting 20µl solution of standard preparations six times into the chromatographic system on different days by maintaining the optimized chromatographic conditions and calculated %RSD of retention time and peak areas for both Metoprolol succinate and Chlorthalidone.

Accuracy
The accuracy of the test method was demonstrated by % recovery across its range by adding a known quantity of the standard to the pre analyzed sample. The recovery was carried out at 80%, 100% and 120% concentration levels using standard addition method and at each level, 3 samples were prepared and total of 9 samples were injected separately into the chromatographic system and the contents were then determined from respective chromatograms. From the results obtained we conclude that the method was accurate.

Limit of Detection (LOD)
Limit of detection is the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the formula.

LOD = $3.3\ S_a/b$
S_a is the standard deviation of intercept
b is the slope of calibration curve

Limit of Quantification (LOQ)
Limit of quantification is the lowest concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantitation can be obtained from linearity curve by applying the following formula.

LOQ = $10\ S_a/\ b$
S_a is the standard deviation of intercept
b is the slope of calibration curve

Robustness
The robustness of the proposed method was determined by analyzing aliquots from homogenous lots by differing physical parameters like mobile organic phase composition, flow rate and column temperature. The standard preparations were injected into the chromatograph at varied conditions of flow rate ± 0.2 ml/min, mobile organic phase ± 10%, mobile phase buffer pH ± 0.2 units and column temperature by $\pm$ 5 ^{0}c. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust.

Stability of the solution and Forced degradation studies:
In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results showed that for the solutions, the retention time and peak area of Metoprolol succinate and Chlorthalidone were remained almost similar (%RSD less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr., which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the developed RP-HPLC method by degrading the sample forcefully under the various stress conditions like acid, base, water, light, heat and peroxide oxidation. The results of the degradation studies are presented.

Acid degradation studies:
Transferred sample quantitatively equivalent to 25 mg of Metoprolol and 6.25 mg of Chlorthalidone in to a 100 ml round bottom (RB) flask, added 50 ml of freshly prepared 0.1 N HCL. After keeping the solution for 10 hrs, filtered and then neutralize the solution up to the volume with 0.1 N NaOH. Further diluted 1.0 ml of the filtrate to 10 ml with a diluent in a 10 ml volumetric flask to obtain final concentration of 50µg/ml & 12.5µg/ml solution of Metoprolol and Chlorthalidone respectively. Then 20 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample**.**

Alkali degradation studies:
Transferred sample quantitatively equivalent to 25 mg of Metoprolol and 6.25 mg of Chlorthalidone in to a 100 ml RB flask, added 50 ml of freshly prepared 0.1 N NaOH. After keeping the solution for 10 hrs, filtered and

then neutralize the solution up to the volume with 0.1 N HCL. Further diluted 1.0 ml of the above solution to 10 ml with a diluent in a 10 ml volumetric flask to obtain final concentration of 50µg/ml & 12.5µg/ml solution of Metoprolol and Chlorthalidone respectively. Then 20 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample**.**

Oxidation degradation studies:
Transferred sample quantitatively equivalent to 25 mg of Metoprolol and 6.25 mg of Chlorthalidone in to a 100 ml RB flask, added 50 ml of freshly prepared 1% Hydrogen peroxide solution. After keeping the solution for 10 hrs on a bench top, filtered and then again diluted 1.0 ml of the filtrate to 10 ml with a diluent in a 10 ml volumetric flask to obtain final concentration of 50µg/ml & 12.5µg/ml solution of Metoprolol and Chlorthalidone respectively. Then 20 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample**.**

Photolytic degradation studies:
Transferred sample quantitatively equivalent to 25 mg of Metoprolol and 6.25 mg of Chlorthalidone on to a clean and dry petri plate. Kept the petri plate in UV Chamber for 10 hrs.Then transferred contents in to a 50 ml volumetric flask, added 30 ml of diluent and sonicate it for 10 minutes and made up to the volume with a diluent. Filtered and again diluted 1.0 ml of the filtrate to 10 ml with a diluent in a 10 ml volumetric flask to obtain final concentration of 50µg/ml & 12.5µg/ml solution of Metoprolol and Chlorthalidone respectively. Then 20 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample**.**

Thermal degradation studies:
Transferred sample quantitatively equivalent to 25 mg of Metoprolol and 6.25 mg of Chlorthalidone on to clean and dry petri plate. Kept the petri plate in an oven at 100°C for 10 hrs. Then transferred the contents in to a 50 ml volumetric flask, added 30 ml of diluent and sonicate it for 10 minutes and made up to the volume with a diluent. Filtered and again diluted 1.0 ml of the filtrate to 10 ml with a diluent in a 10 ml volumetric flask to obtain final concentration of 50µg/ml & 12.5µg/ml solution of Metoprolol and Chlorthalidone respectively. Then 20µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample**.**

Neutral degradation studies:
Stress testing under neutral conditions was studied by refluxing the standard stock solution of Metoprolol and Chlorthalidone on water bath for 6 hrs at a temperature of 60ºc. For HPLC study, the resultant solution was suitably diluted with a diluent in a 10 ml volumetric flask to obtain final concentration of 50µg/ml & 12.5µg/ml solution of Metoprolol and Chlorthalidone respectively. Then 20 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample**.**

RESULTS AND DISCUSSION

From this study, it was found that a simple, precise, accurate, sensitive and efficient stability indicating RP-HPLC method has been developed and validated for simultaneous estimation of Metoprolol and Chlorthalidone in bulk and combined tablet dosage form. Chromatographic separation was carried out using mobile phase composed of phosphate buffer (adjusted to pH 5.5 with dilute OPA) and acetonitrile in the ratio of 55:45%v/v on Inertsil ODS C_{18} (250 x 4.6 mm, 5µ particle size) column at a flow rate 0.8 ml/min using PDA detection at 219 nm. The retention time was found to be 3.763 min and 4.924 min for Metoprolol and Chlorthalidone respectively. The Isobestic point of Metoprolol succinate and Chlorthalidone was found to be 219.2 nm (as shown in figure 3) after scanning 10µg/ml working standard solutions of both Metoprolol succinate and Chlorthalidone in the UV region of 200-400 nm against reagent blank and was utilized for HPLC method development. System suitability chromatogram as shown in figure 4 and results are shown in table 1. Linearity was evaluated in the concentration range of 12.5-75 µg/ml for Metoprolol and 3.125-18.75µg/ml for Chlorthalidone. The calibration curves were described by the equation y = 79331.9x-6434.2 and y = 77857.25x +1977.64 with correlation coefficient of 0.99997 for Metoprolol succinate and Chlorthalidone respectively as shown in figure 5 and figure 6 respectively. The standard and sample chromatograms in the specifity studies are shown in figure 7, figure 8 and figure 9. Accuracy data as shown in table 2. The validation summary parameters and assay results obtained from the marketed formulation are shown in table 3 and table 4. The results of robustness studies are shown in table 5 and table 6. The %RSD in precision, accuracy and robustness studies were found to be less than 2%, indicating that the method was precise, accurate and robust. The stress testing chromatograms for both Metoprolol succinate and Chlorthalidone are shown from figure 10 to figure 15 and results are shown in table 7 and table 8.

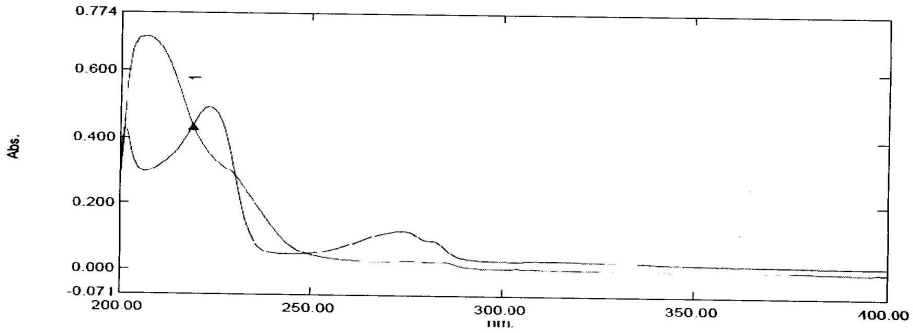

Fig. 3: Isobestic point of Metoprolol succinate and Chlorthalidone (1=219.2 nm)

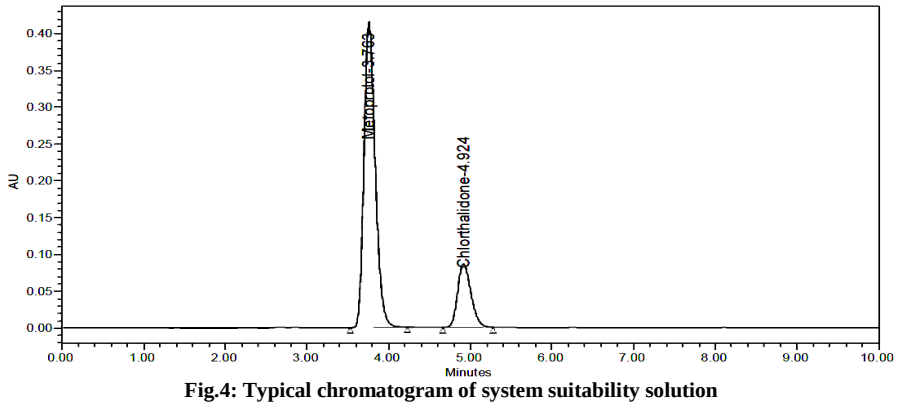

Fig.4: Typical chromatogram of system suitability solution

Table 1: System suitability results

S.No. S.No	System suitability parameters	Metoprolol succinate	Chlorthalidone
1	Tailing factor (T_f)	1.18	1.16
2	Resolution (Rs)	4.12	
3	Retention time (Min)	3.763	4.924
4	Theoretical plates (N)	3572	4345
5	Peak Area	3977745	978651

Table 2: Accuracy (recovery) studies

Sample	% Concentration Level	Peak Area*	Amount added (mg/tab)	Amount recovered (mg/tab)	Mean % Recovery *± SD
Metoprolol succinate	80	3179604	20	20.00	99.98 ±0.28
	100	3973062	25	24.99	99.94±0.37
	120	4737591	30	29.97	99.32± 0.44
Chlorthalidone	80	780185	5	4.99	99.72±0.54
	100	976466	6.25	6.24	99.84±0.31
	120	1167930	7.50	7.46	99.52 ±0.42

**Mean of three determinations*

Linearity:

R^2 values were found to be 0.99997 and 0.99997 and regression equation y = 79331.9x-6434.2 and y = 77857.25x +1977.64 for Metoprolol succinate and Chlorthalidone respectively.

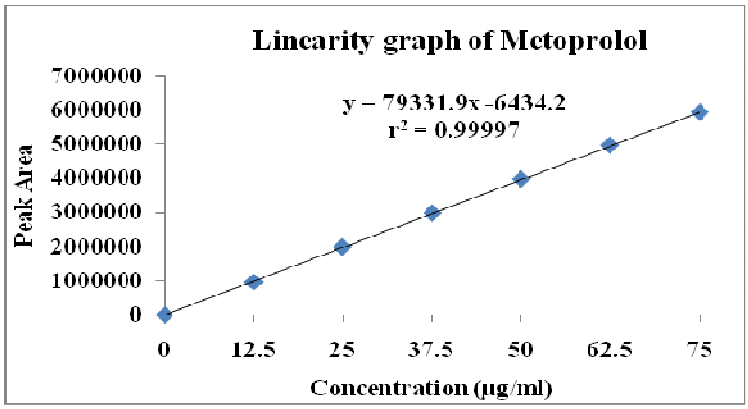

Fig. 5: Linearity Graph of Metoprolol succinate (12.5-75 µg/ml)

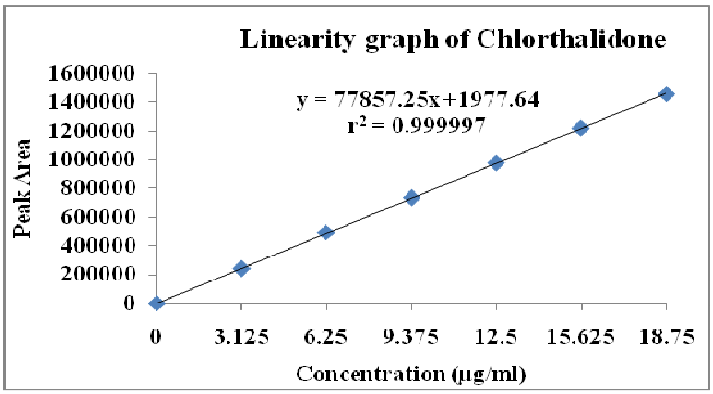

Fig. 6: Linearity Graph of Chlorthalidone (3.125-18.75 µg/ml)

Table 3: Validation summary Parameters of the proposed RP-HPLC Method

Parameter	Metoprolol succinate	Chlorthalidone
Regression equation	y = 79331.9x-6434.2	y = 77857.25x +1977.64
Correlation coefficient	0.99997	0.99997
LOD (µg/ml)	0.624	0.127
LOQ (µg/ml)	2.26	0.442
System precision (% RSD)	0.22	0.27
Method precision (% RSD)	0.18	0.35
Inter-day precision (% RSD)	0.17	0.12

Table 4: Results of Assay in Marketed Formulation

Brand	Drug	Standard peak area	Sample peak area	Labelled amount (mg/tab)	Amount found (mg/tab)	% Assay	%RSD*
Vinicor-D	Metoprolol succinate	3977254	3971328	25	24.94	99.76%	0.18
	Chlorthalidone	977603	976965	6.25	6.24	99.86%	0.26

**Mean of three determinations*

Specificity studies:

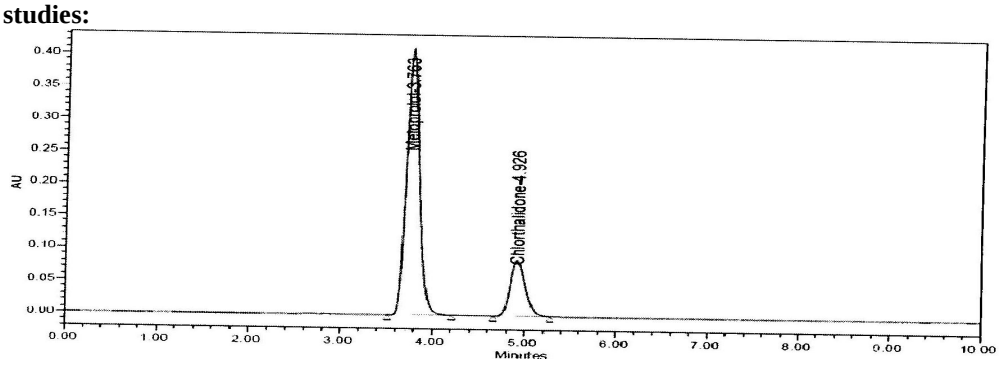

Fig.7: Typical chromatogram of standard

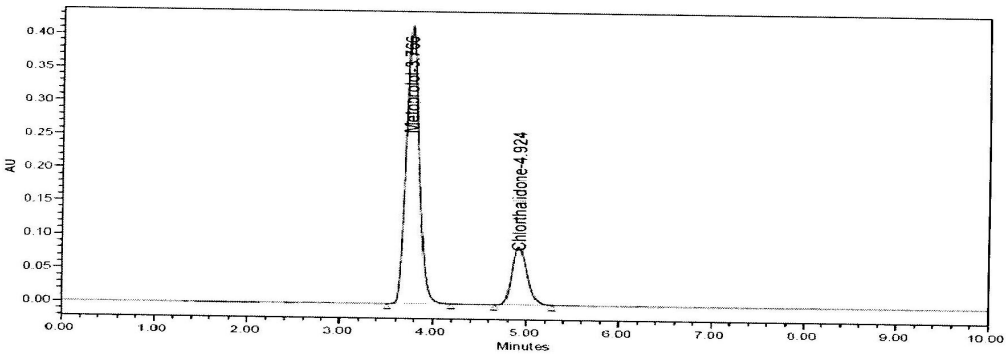

Fig.8: Typical chromatogram of sample 1

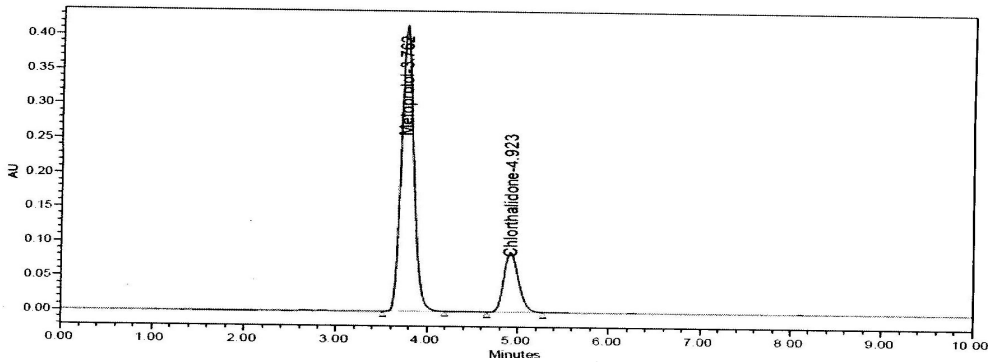

Fig.9: Typical chromatogram of sample 2

Robustness:

The developed method was robust with deliberate changes in variation of mobile organic phase composition, flow rate and temperature for both Metoprolol succinate and Chlorthalidone respectively.

Table 5: Results of Robustness Study of Metoprolol

S.No.	Parameter	Change Level	Metoprolol			
			Rt (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (±0.2ml/min)	0.6	4.684	4974306	1.16	3870
		1.0	3.144	3307259	1.17	3688
2.	Mobile organic phase composition (±10%v/v/v)	65:35	3.918	4273664	1.15	3531
		45:55	3.452	3725698	1.18	3947
3.	Column temperature (±5°C)	25 ^{0}C	3.746	3968974	1.17	3629
		35 ^{0}C	3.785	3983948	1.16	3458

Table 6: Results of Robustness Study of Chlorthalidone

S.No.	Parameter	Change Level	Chlorthalidone			
			Rt (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (±0.2ml/min)	0.6	6.116	1227253	1.18	4430
		1.0	4.104	818621	1.16	3887
2.	Mobile organic phase composition (±10%v/v/v)	65:35	5.874	1048736	1.19	4118
		45:55	4.327	968572	1.17	4625
3.	Column temperature (±5°C)	25 ^{0}C	5.198	984570	1.15	4987
		35 ^{0}C	4.945	987738	1.16	4310

Forced degradation studies:

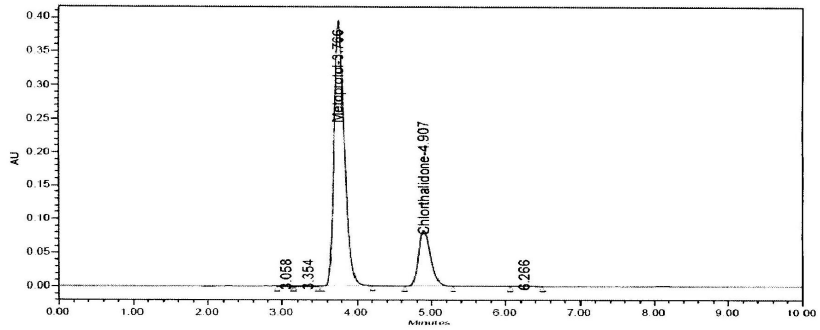

Fig.10: Chromatogram of Acid hydrolysis

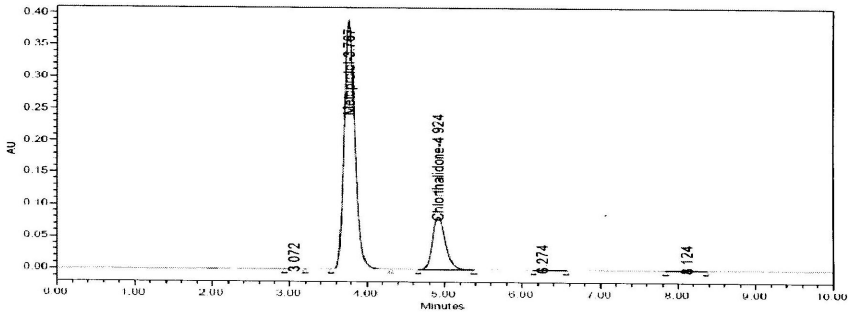

Fig.11: Chromatogram of Base hydrolysis

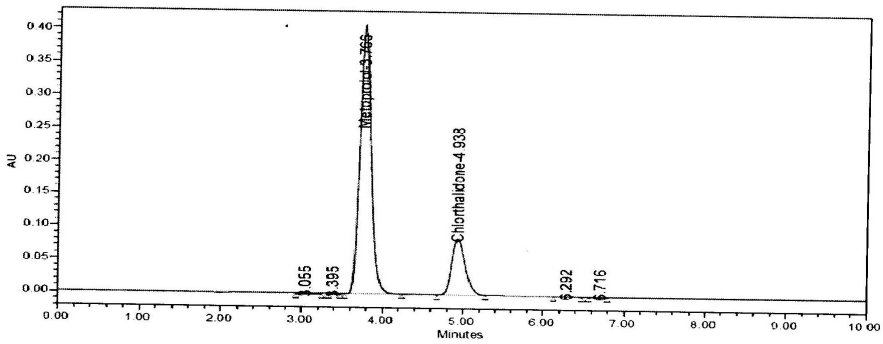

Fig.12: Chromatogram of Oxidation (peroxide) degradation

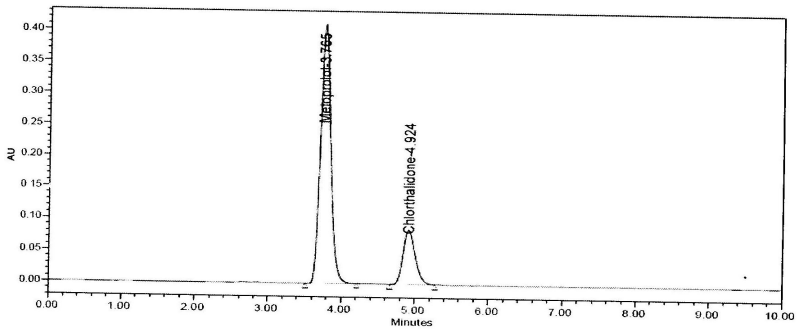

Fig.13: Chromatogram of thermal degradation

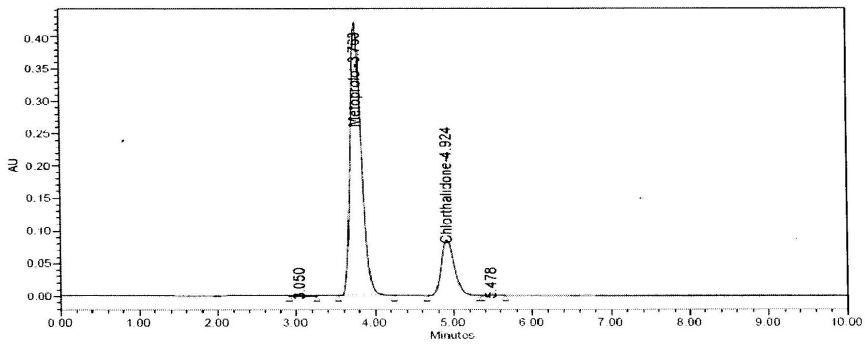

Fig.14: Chromatogram of UV Exposure

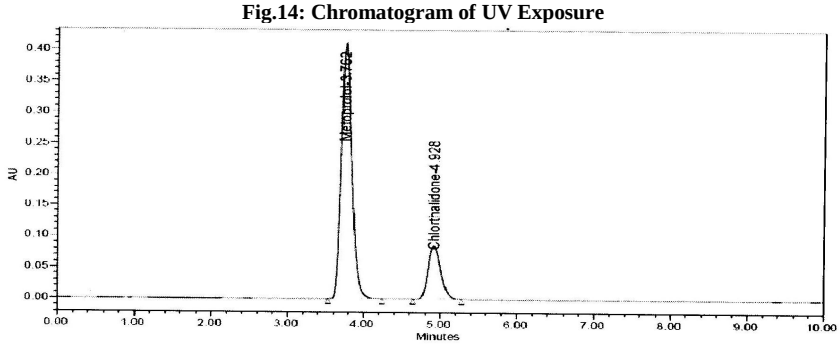

Fig.15: Chromatogram of Neutral degradation

Table 7: Degradation Study of Metoprolol succinate

S.No.	Stress Condition	Retention time (min)	Peak Area	System Suitability parameters		degradation % Assay	% Net degradation
				USP Tailing	USP Plate count		
1	Acid Hydrolysis	3.766	3783252	1.15	3561	94.84	4.92
2	Base Hydrolysis	3.767	3740181	1.18	3516	93.76	6.10
3	Peroxide degradation	3.466	3835638	1.16	3485	96.15	3.61
4	Thermal degradation	3.765	3970452	1.18	3678	97.20	2.62
5	UV Exposure	3.763	3853607	1.16	3691	96.60	3.16
6	Neutral degradation	3.762	3908752	1.18	3565	97.52	2.35

Table 8: Degradation Study of Chlorthalidone

S.No.	Stress Condition	Retention time (min)	Peak Area	System Suitability parameters		degradation % Assay	% Net degradation
				USP Tailing	USP Plate count		
1	Acid Hydrolysis	4.907	9	1.14	4248	95.74	4.12
2	Base Hydrolysis	4.924	925379	1.16	4266	94.45	5.41
3	Peroxide degradation	4.938	928144	1.16	4252	94.73	5.13
4	Thermal degradation	4.924	965871	1.15	4286	97.14	2.78
5	UV Exposure	4.924	952739	1.14	4247	97.24	2.62
6	Neutral degradation	4.928	958426	1.16	4345	97.46	2.47

CONCLUSION

From this study, it is concluded that the proposed Stability indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Metoprolol succinate and Chlorthalidone in bulk & combined tablet dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

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