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Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Metoprolol Succinate and Chlorthalidone in Bulk and in Tablet Dosage Form

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ABSTRACT

RP-HPLC method has been developed along with stability indicating attribute for simultaneous estimation of Metoprolol Succinate and Chlorthalidone in bulk and in tablet dosage form with minimized drug extraction steps. The chromatographic analysis was performed isocratically by using Oyster ODS3, 150-4.6 mm column having particle size of 5 μm (Merck & Co.) as stationary phase maintained at ambient temperature (about 25°C) with 1.0 mL/min of flow rate and 20 mM phosphate buffer having pH 2.3 (adjusted with10% orthophosphoric acid) and Acetonitrile (650:350, v/v) as Eluent at wavelength 225 nm. Individual drug substances as well as combination drug product was subjected to acid, alkali, oxidative, photolytic, thermal and humidity degradation, the peaks due to degraded product were significantly separated out from active analytes peak. The method was validated for the specificity, linearity, detection limit, quantitation limit, precision, accuracy, robustness and solution stability as per ICH guidelines and successfully used for regular analysis.

Keywords: Metoprolol Succinate, Chlorthalidone, Forced Degradation, Validation, Solution stability, RP-HPLC.

INTRODUCTION

Metoprolol Succinate (MTL) is chemically, Bis[(2RS)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol] butanedioate which is a white or almost white, crystalline powder and official in United State Pharmacopeia, Indian Pharmacopoeia and British Pharmacopeia. It is categorized as Beta-adrenoceptor antagonist.

Chlorthalidone (CTD) is chemically, 2-Chloro-5-[(1RS)-1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl]benzenesulphonamide which is white or yellowish-white powder and official in United State Pharmacopeia, Indian Pharmacopoeia and British Pharmacopeia. It is categorized as diuretic. The chemical structure of Metoprolol Succinate and Chlorthalidone are shown in Figure 1.[1-3]

The diuretics and beta-adrenergic blockers are drugs of first choice in the treatment of essential arterial hypertension. But in many cases, monotherapy of diuretic or beta-blocker may fail to control the blood pressure satisfactorily, hence the fixed dose combination therapy of Metoprolol and Chlorthalidone used for the management of arterial hypertension or high blood pressure, a leading cause of death.[4]

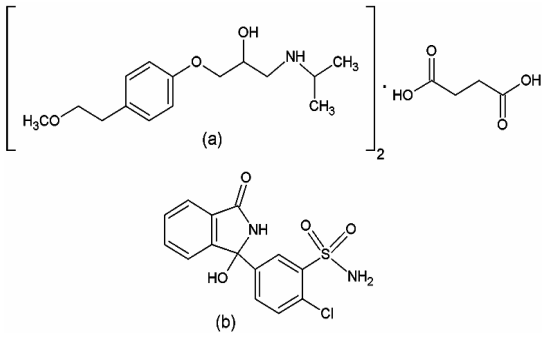

Fig. 1. Structure of (a) Metoprolol Succinate; (b) Chlorthalidone

Survey of literature reveals that few methods are available for simultaneous estimation of MTL and CTD by UV-Spectrophotometric[5-6] and HPLC[7-11] techniques. The existing methods have one of the drawbacks like time consuming procedures, long run time, less sensitivity and low resolution. Among all these reported methods, two methods reported for assay of Metoprolol Succinate and Chlorthalidone in which forced degradation study was performed but with poor experimental design. In addition to this, stability of mobile phase preparation, standard and sample solution along with some robustness parameters need to be performed. Hence, an attempt has been made to perform forced degradation study with proper experimental design on individual drug substances and combination drug product to develop a validated RP-HPLC method with stability indicating attribute for the simultaneous estimation of MTL and CTD which is more simple, rapid, sensitive, accurate, precise and robust enough.

To understand the inherent stability characteristics of active component(s), stress testing needs to be carried out.[12] Impurities and/or related substances are generated from the manufacturing process and/or degradation products from improper storage or handling or as metabolites which could be active, inactive or even toxic that will significantly impact the results with respect to quality, safety, and efficacy. A good stability-indicating methods having capability to separate the active component significantly from its degradation products or impurities.[13-16]

MATERIALS AND METHODS

Chemicals/Materials/Reagents

Metoprolol Succinate pure drug substance gift sample supplied by Ajanta Pharma Ltd., Paithan-India (Batch no.: 20MS00079, Potency: 99.68%) and Chlorthalidone pure drug substance gift sample supplied by Alkem Laboratories Ltd., Mumbai-India (Batch no.: HLm0340720, Potency: 99.65%). All the chemicals like Potassium Dihydrogen Phosphate (Batch no.: H14A/1514/1306/53, Make: SD Fine Chem Ltd.), Ortho-Phosphoric acid (Batch no.: 2467211117, Make: Research Lab Fine Chem Industries), Acetonitrile (Batch no.: 1038350516, Fischer Scientific India Pvt. Ltd.), Sodium Hydroxide (Batch no.: DH6D662478, Make: Merck & Co.), 6% v/v Hydrogen Peroxide (Batch no.: MCM-1171, Make: Molychem), Hydrochloric acid (Batch no.: CK6C660816, Make: Merck & Co.), Water etc of HPLC grade or equivalent grade were used during the experiments. Metoprolol Succinate and Chlorthalidone Tablets 47.5/12.5 mg (Revelol®–CH 50/12.5)-each uncoated bilayared tablets contains 47.50 mg Metoprolol Succinate which equivalent to 50 mg Metoprolol Tartrate and 12.5 mg Chlorthalidone was purchased from local drug shop (Batch no.: GYD039001AK, Make: IPCA Laboratories Ltd., Mumbai-India).

Instruments

Analytical weighing balance (Make: Citizon, Model: CY204) was used for weighing of the materials. Sonication was done by using Digital Ultrasonic Cleaner (Make: Labman Scientific Instruments, Model: LMUC-3). Digital pH meter (Make: Labtronic Laboratory Instruments, Model: LT49) was utilized for the checking of solution pH. The Stability Chamber (Make: Labline Stock Centre, Model: GMP), Photostability Chamber (Make: S R Lab Instruments India Pvt. Ltd., Model: SRL-PHSC-11-A) and Hot Air Oven (Make: Bio-Technics India, Model: BTI-29) were used to perform the forced degradation study. Refrigerator (Make: LG, Model: GL-A282SPZL) was used during solution stability study. Water purification system (Make: Analytical

Technologies Limited, Model: WPS211) was used for collecting the Ultrapure water for the experiment. The method was developed on Oyster ODS3, 150-4.6 mm column having particle size of 5 μm (P/N: S670153, Make: Merck & Co.) column connected to a HPLC system (Make: Shimadzu, Model: SCL-10Avp) equipped with UV detector having rheodyne sample injection port with 20 µL loop. The chromatographic instrument was controlled by LC Solution software version 1.25 and same used for the chromatographic data handling.

Chromatographic conditions

The details of chromatographic conditions maintained for analysis during the experimental work are given in Table 1.

Table 1: Chromatographic conditions

Parameters		Description
Type of system	:	HPLC with UV detector or equivalent
Mobile Phase (Eluent)	:	20 mM phosphate buffer with pH 2.3 and Acetonitrile (650:350, v/v)
Column	:	Oyster ODS3, 150-4.6 mm column having particle size of 5 μm (P/N: S670153, Make: Merck & Co.)
Detection Wavelength	:	225 nm
Flow rate	:	1.0 mL/min
Volume of injection	:	20 μL
Temperature of column	:	Ambient (about 25°C)
Pump mode	:	Isocratic
Run time	:	8 min
Diluent/blank	:	Mobile Phase used as diluent

Preparation of 20 mM phosphate buffer pH 2.3

Weighed 2.72 g Potassium Dihydrogen Phosphate (PDP) and transferred into 1000 mL Water, dissolved with 10 min of sonication. The pH 2.3 adjusted with 10% Ortho-Phosphoric acid solution and filtered by using 0.45 μm Nylon membrane filter (Cat no.: HNNX0902XXXX104, Make: Advanced Microdevices Pvt. Ltd.).

Preparation of mobile phase

Mixed 350 mL of Acetonitrile with 650 mL of 20 mM phosphate buffer having pH 2.3 and degassed by 10 min of sonication.

Preparation of standard solution

Weighed 47.5 mg MTL and 12.5 mg of CTD standard and transferred into a 250 mL dry volumetric flask. Added 175 mL diluent and sonicated for 10 min with intermediate shaking to dissolve. After sonication, allowed to attain room temperature and with diluent, made up to the mark and mixed well (Concentration of Metoprolol Succinate = 190 µg/mL; Concentration of Chlorthalidone = 50 μg/mL).

Standard solution prepared in duplicate to confirm the suitability of standard.

Preparation of sample solution

Average weight of Metoprolol Succinate and Chlorthalidone Tablets 47.5/12.5 mg (Revelol®–CH 50/12.5) was determined from weight of 20 tablets. Powdered these tablets by using mortor and pestle, and then transferred 376.5 mg (equivalent to MTL 47.5 mg and CTD 12.5 mg) of this fine powder into a 250 mL dry volumetric flask. Added 175 mL diluent and sonicated for 25 min with intermediate shaking. After sonication, allowed to attain the room temperature and with diluent, made up to the mark and mixed well. Finally, filtered by using Whatman filter paper (Cat No.: 1001-125, Make: GE Healthcare UK Ltd.) by discarding initial 5 mL of the filtrate and used as sample solution for assay.

Method validation

The proposed chromatographic method was validated as per the ICH guideline Q2(R1).[17]

Specificity

For specificity, interference of the blank solution at the retention time of MTL and CTD peak were checked. Also, specificity were studied in forced degradation studies with extended run time to the twice of actual run time which ensure that no late eluting degradant peaks, as elution mode is isocratic. In this study, the forced degradation was performed by exposing both drug substances individually and sample of drug product (Revelol®–CH 50/12.5) with known concentration to different stress conditions like acid degradation (5 N Hydrochloric acid, 3 h at room temperature), alkali degradation (5 N Sodium hydroxide, 3 h at room temperature), oxidative degradation (6% v/v Hydrogen Peroxide, 3 h at room temperature), thermal degradation (dry heat at 90^{0}C for 24 h in hot air oven), photolytic (UV light for 24 h in Photostability chamber) and humidity degradation (75% relative humidity for 48 h in stability chamber). Similarly, acid, alkali and oxidative stressed blank solutions were prepared without active component to check any interference at retention time of active analytes peaks. However, samples of stress

degradation were analyzed by using the proposed chromatographic method and mass balance results (%assay + %degradant) was calculated for all the stressed samples against standard solution and compared with unstressed sample.

System Suitability and System Repeatability

The system suitability parameters like tailing factor, peak area, retention time, resolution and theoretical plates count were determined from 1st injection of standard solution. The systems repeatability parameters are determined by injecting the five replicates of first standard solution and single replicate of second standard solution in the chromatographic system and further determining the %RSD for standard 1 and %relative difference for standard 2.

Linearity

Linearity was demonstrated at five different concentration levels prepared from standard stock solution (Concentration of Metoprolol Succinate = 948.9536 μg/mL and Chlorthalidone = 248.1285 μg/mL). It was performed from 60% to 140% of the nominal working concentration in the range of 113.8744 - 265.7070 μg/mL and 29.7754 - 69.4760 μg/mL for MTL and CTD respectively. The linearity graph was plotted for concentration versus peak area response and determined the squared correlation coefficient (R^2).

Detection Limit and Quantitation Limit

The detection limit (DL) and quantitation limit (QL) of MTL and CTD were determined based on the standard deviation (residual value) of response and the slope method. As per ICH guideline, it was determined from calibration curve of MTL and CTD by using below mentioned formulae.

$DL=(3.3 \times \sigma)/S$ and $QL=(10 \times \sigma)/S$

Where, σ = the standard deviation of the response; S = the slope of the calibration curve

Accuracy

Accuracy were assessed by triplicate analyses of sample containing placebo mixture with Metoprolol Succinate and Chlorthalidone at three concentrations 60%, 100% and 140% of the nominal working concentration. At every concentration level, a triplicate samples were prepared and each sample was injected once, and the average recovery for triplicate samples at each concentration level was calculated.

Precision

For assay determination of MTL and CTD, precision study performed by using homogeneous samples.

Method Repeatability

Method Repeatability was demonstrated by injecting six sample preparations of MTL and CTD Tablets 47.5/12.5 mg (Revelol®–CH 50/12.5) using batch no. GYD039001AK (Make: IPCA Laboratories Ltd., Mumbai-India) as per developed method. Assay sample preparation was made on 6 replicate samples and calculated the %Assay, %RSD and 95 %confidence interval (95% CI). Also, the system suitability and the system repeatability results were determined.

Intermediate Precision

Intermediate Precision was demonstrated from six determinations of the same sample of MTL and CTD Tablets 47.5/12.5 mg (i.e. batch, storage conditions, container, etc) tested for Method Repeatability by different analyst on different day. Assay sample preparation was made on 6 replicate samples and calculated the %Assay, %RSD, 95% confidence interval (95% CI) and compares the average results obtained in the Method Repeatability and Intermediate Precision study. Also, the system suitability and the system repeatability results were determined.

Robustness

The method robustness was demonstrated by doing conscious changes in method parameters. Filter compatibility was demonstrated for MTL and CTD Tablets 47.5/12.5 mg (Revelol®–CH 50/12.5) using three sample preparations. Each sample solution was divided into three parts. First part was filtered by using Whatmann filter (Cat no. 1001-125, Make: GE Healthcare UK Ltd.) by discarding initial 5 mL of the filtrate as per method. The second part was filtered by using 0.45 μm PVDF Syringe filter (Cat no. SYVF0602MNXX104, Make: Advanced Microdevices Pvt. Ltd.) by discarding initial 5 mL of the filtrate and third part was filtered by using 0.45 μm Nylon Syringe filter (Cat no. SYNN0602MNXX104, Make: Advanced Microdevices Pvt. Ltd.) by discarding initial 5 mL of the filtrate and used as the sample solution, the %assay and %relative difference were calculated.

The extraction efficiency of method was established by doing conscious changes into sonication time for sample preparation from 20 min to 30 minutes. Change in sonication time for sample preparation was checked with three replicate sample preparations of MTL and CTD Tablets 47.5/12.5 mg (Revelol®–CH 50/12.5) for each changed condition, the %assay and %relative difference were calculated.

As part of robustness study, deliberate change in chromatographic parameters with respect to change in flow rate (± 0.1 mL/min) from 0.9 mL/min to 1.1 mL/min; change in the organic composition of mobile phase (± 10%) from 650:315, v/v to 650:385, v/v; change in pH (± 0.1) of mobile phase buffer from pH 2.2 to pH 2.4; change in the quantity of Potassium Dihydrogen Phosphate for mobile phase buffer (± 10%) from 2.448 g/1000 mL to 2.992 g/1000 mL and each changed condition impact on the method was assessed. The system suitability and system repeatability results were checked for each changed condition.

Solution Stability

The stability of standard solutions carried out on two preparations and evaluated after day 1 and day 2, storage at room temperature and in refrigerator (2-8°C). The stored standard solution results were compared with freshly prepared standard solution; the %relative difference was calculated.

The stability of sample solution carried out on triplicate sample solutions and evaluated after day 1 and day 2, storage at room temperature and in refrigerator (2-8°C) as per test method. Results of stored sample solution were compared with initial sample solutions and the %relative difference between the %assays was calculated.

Stability of mobile phase preparation was evaluated at room temperature after day 1 and day 2. The results for change in appearance, system suitability and system repeatability were checked during evaluation of mobile phase stability.

Range

Linearity as well as accuracy for Metoprolol Succinate and Chlorthalidone was checked from 60 to 140% of the nominal working standard solution. The method range is checked, based on suitable linearity, accuracy and precision results.

RESULT AND DISCUSSION

Method Development and Chromatographic Conditions Optimization

The consideration of specificity, linearity, accuracy, precision, robustness and solution stability parameters for development and validation of stability indicating method for MTL and CTD in bulk and in tablet dosage form. The Eluent was selected and optimized after use of a number of changed compositions followed by optimization of detection wavelength and flow rate. Method optimization performed by using the columns like Oyster ODS3, 150 - 4.6 mm column having particle size of 5 μm (P/N: S670153, Make: Merck & Co.) and ODS Hypersil, 250 - 4.0 mm column having particle size of 5 μm (P/N: 30105-254030, Make: Thermo Scientific). The organic modifiers like acetonitrile and methanol were used with 20 mM Phosphate buffer at a different pH level as 2.3, 2.5 and 3.0 to obtain best peak with optimum resolution.

Finally, the Eluent comprised in the ratio of 650:350, v/v of 20 mM phosphate buffer pH 2.3 ± 0.05 (adjusted with 10% orthophosphoric acid) and Acetonitrile was selected for simultaneous estimation of MTL and CTD because it retain both the peak efficiently in short time with satisfactory resolution, tailing factor (symmetry factor) and plate count (number of theoretical plates). Assay was carried out with 1.0 mL/min flow rate with ambient column temperature (about 25°C) and response recorded by UV detector at 225 nm. All quantitative assay calculations of MTL and CTD were done based on peak area response.

Method Validation

Specificity

Specificity was established by demonstrating that, there is no blank interference with Metoprolol and Chlorthalidone peak (Figure 2).

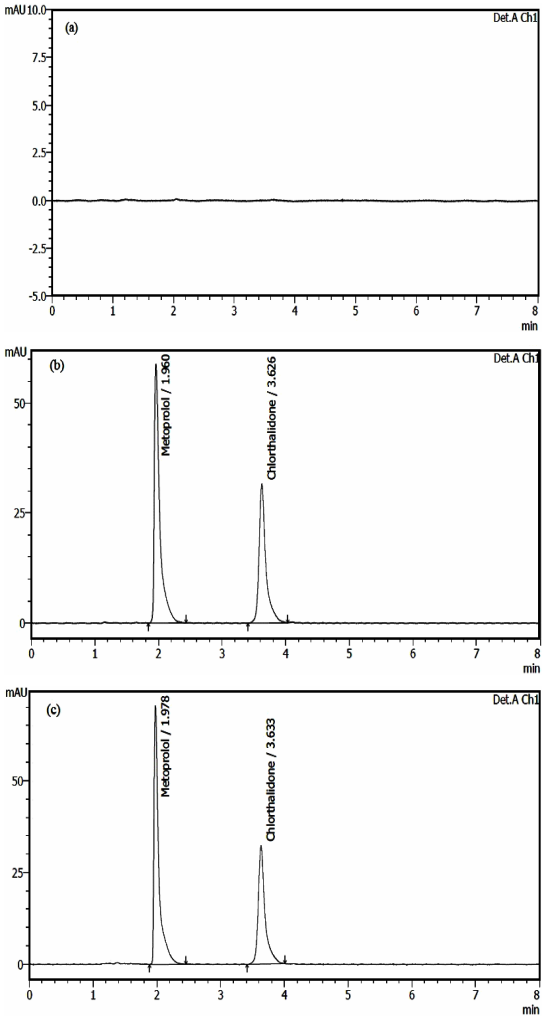

Fig. 2. Chromatograms of (a) Blank; (b) Standard; (c) Sample

The forced degradation study was performed and no interference was observed from the peaks of degradant with retention time of MTL and CTD. The mass balance results (%assay + %degradant) was calculated for all the stressed samples against standard solution and compared with unstressed sample. Mass balance data for MTL and CTD in their respective API solution clearly demonstrated that there is no significant effect of stressed conditions on response. Hence, the forced degradation studies showed that MTL and CTD API were stable to acid, alkali, oxidative, thermal, photolytic and humidity stressed degradation as no additional degradant peak detected.

Mass balance data for MTL and CTD in the sample solution clearly demonstrated that the response of both active components decreased in thermal stressed samples along with increase in the response of degradant peaks. In thermal stressed sample, the major degradant observed at 1.738 min and 5.710 min for MTL and CTD respectively. Hence, the forced degradation studies showed that both MTL and CTD in sample solution was stable to acid, alkali, oxidative, photolytic and humidity stressed degradation while susceptible to thermal stress degradation.

The forced degradation study results are summarized in Table 2. The chromatograms of stressed samples (thermal stressed) were showed degradation product peak significantly distinguishable from the drugs peak, indicating that the method is specific (Figure 3).

Table 2: Summarized results of forced degradation study

Name of the sample	Condition	Drug Substance						Drug Product					
		%Assay		%Total degradation		%Mass Balance		%Assay		% Total degradation		%Mass Balance	
		MTL	CTD	MTL	CTD	MTL	CTD	MTL	CTD	MTL	CTD	MTL	CTD
Unstressed	As per test method	99.35	98.62	NTD	NTD	99.35	98.62	97.58	99.29	NTD	NTD	97.58	99.29
Acid stressed	5N HCl for 3 h at RT	99.68	99.72	NTD	NTD	99.68	99.72	98.80	97.80	NTD	NTD	98.80	97.80
Alkali stressed	5N NaOH for 3 h at RT	97.73	100.16	NTD	NTD	97.73	100.16	98.92	98.48	NTD	NTD	98.92	98.48
Oxidative stressed	6 % H_2O_2 for 3 h at RT	98.73	98.70	NTD	NTD	98.73	98.70	100.05	98.78	NTD	NTD	100.05	98.78
Thermal stressed	90°C for 24 h in Oven	98.80	99.06	NTD	NTD	98.80	99.06	73.30	76.12	4.39	6.25	77.79	82.37
Photolytic stressed	UV-light for 24 h	99.98	98.88	NTD	NTD	99.98	98.88	97.61	98.07	NTD	NTD	97.61	98.07
Humidity stressed	75% RH for 48 h	98.06	97.83	NTD	NTD	98.06	97.83	98.17	98.31	NTD	NTD	98.17	98.31

NTD = Not detected; RT = Room temperature; RH = Relative humidity

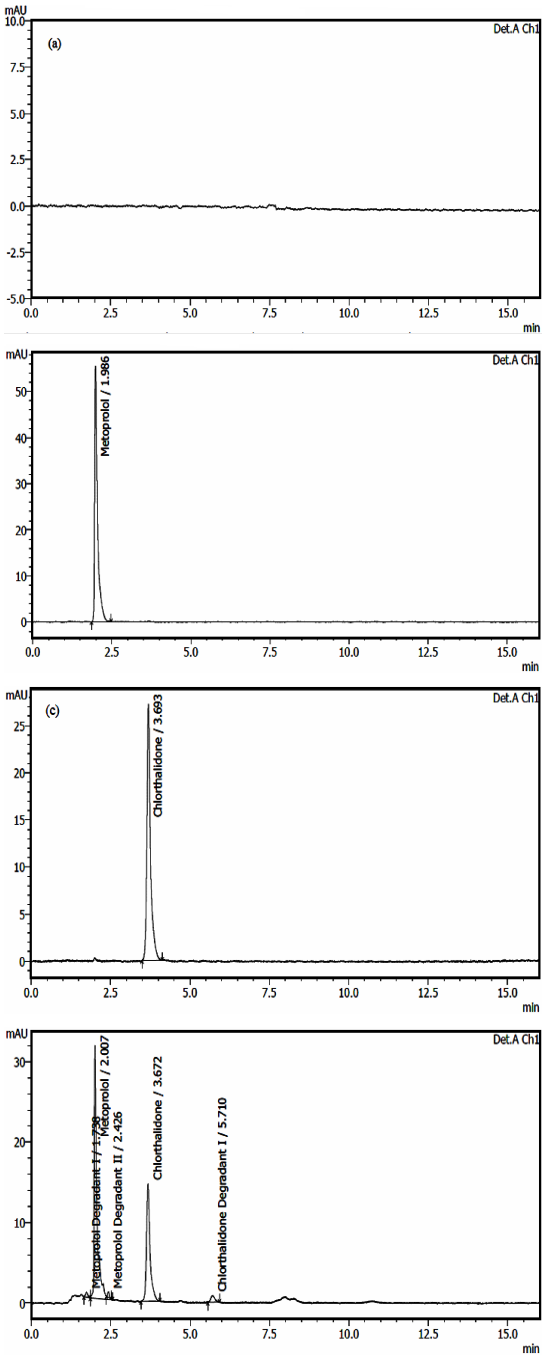

Fig. 3. Chromatogram of (a) unstressed blank; (b) thermal stressed Metoprolol Succinate; (c) thermal stressed Chlorthalidone; (d) thermal stressed sample

System Suitability and System Repeatability

The reproducibility attribute of any chromatographic system has measured through system suitability and system repeatability parameters (Table 3).

Table 3: System suitability and system repeatability results

Parameters	MTL	CTD	Acceptance criteria
Retention time (minutes)	1.960	3.626	–
USP tailing factor (symmetry factor)	1.59	1.28	0.8 – 2.0
USP plate counts (number of theoretical plates)	3587	7526	> 2000
USP resolution	–	11.18	> 6.0
%RSD of five replicate injections of standard 1	0.29	0.52	≤ 2.0 %
The % relative difference between two standards	0.34	0.85	≤ 2.0 %

Linearity

The method was found to be linear for MTL and CTD from 60% to 140% of the nominal working concentration in the range of 113.8744 - 265.7070 μg/mL and 29.7754 - 69.4760 μg/mL with 0.999 as squared correlation coefficient (R^2) in both cases. Results of linearity showed an excellent linear relationship in the studied concentration range for MTL and CTD, indicating the fitness of method for analysis. Linearity study results are summarized in Table 4 and plot is shown in Figure 4.

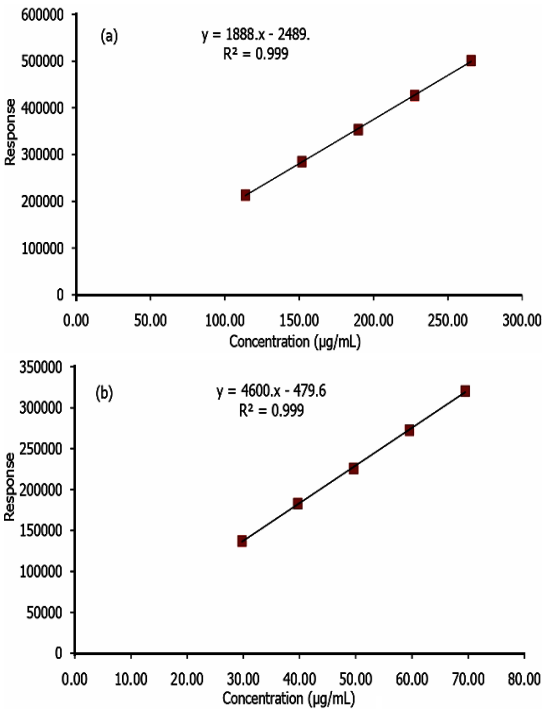

Fig. 4. Linearity graph of (a) Metoprolol Succinate; (b) Chlorthalidone

Table 4: Summarized linearity study results

Linearity Level(%)	Concentration (μg/mL)		Response (peak area)	
	MTL	CTD	MTL	CTD
60	113.8744	29.7754	213553	137204
80	151.8326	39.7006	284907	182803
100	189.7907	49.6257	353591	225785
120	227.7489	59.5508	426448	272697
140	265.7070	69.4760	501215	320543
Squared correlation coefficient (r^2); NLT 0.995			0.999	0.999
(Y-intercept/response at 100% standard concentration) x 100; NMT 3.0%			0.70	0.21

Detection Limit and Quantitation Limit

The detection limit was 3.0169 μg/mL for MTL and 0.9908 μg/mL for CTD respectively, indicating that even small quantities of the MTL and CTD can be detected.

The quantitation limit was 9.1420 μg/mL for MTL and 3.0024 μg/mL CTD respectively, indicating that even small quantities of the MTL and CTD can be determined.

Accuracy

The mean %recovery results obtained from triplicate samples at each level were found to be 100.09, 98.76 and 99.41% for Metoprolol Succinate and 98.79, 99.25 and 99.16% for Chlorthalidone at 60%, 100% and 140% of nominal working concentration respectively, indicating that the method is accurate and showing that no interference from the excipients in the estimation (Table 5).

Table 5: Summarized results for accuracy study

Accuracy level (%)	Concentration (μg/mL)		Mean% Recovery*		%RSD	
	MTL	CTD	MTL	CTD	MTL	CTD
60	114	30	100.09	98.79	1.29	0.55
100	190	50	98.76	99.25	1.42	0.83
140	266	70	99.41	99.16	0.57	1.09

*Mean of three replicates

Precision

Method repeatability and intermediate precision results were shown that, the less than 2.0 %RSD values for MTL and CTD, signifying the method is precise and reproducible (Table 6).

Table 6: Summarized %assay results of precision study

Sample no	Method repeatability		Intermediate precision	
	MTL	CTD	MTL	CTD
1	99.37	100.01	100.42	98.68
2	99.47	98.74	99.01	99.34
3	98.11	99.64	100.11	99.05
4	100.52	98.86	99.17	101.72
5	99.29	99.83	101.94	98.34
6	99.25	97.72	100.74	100.74
Average	99.34	99.13	100.23	99.64
%RSD	0.78	0.87	1.08	1.31
95% CI	99.09 – 99.59	98.85 – 99.41	99.88 – 100.58	99.21 – 100.07
%Relative Difference	–	–	0.89	0.51

Robustness

Results obtained from sample filtered by using 0.45 μm Nylon Syringe filter was meet acceptance criteria for %relative difference while 0.45 μm PVDF Syringe filter does not meet acceptance criteria for %relative difference (should be not more than 3.0%) with results obtained from sample filtered through Whatmann filter paper (as per method). Results of 0.45 μm Nylon Syringe filter are acceptable whereas results 0.45 μm PVDF Syringe filter are not acceptable. Hence, in addition to Whatmann filter paper, only 0.45 μm Nylon Syringe filter are useful for assay samples (Table 7).

Table 7: Robustness results for filter compatibility

Filter	Sample no	Metoprolol Succinate		Chlorthalidone	
		%Assay	% Relative Difference	%Assay	%Relative Difference
Whatmann filter (As per method)					
	1	100.52	NA	98.86	NA
	2	99.29	NA	99.83	NA
	3	99.25	NA	97.72	NA
0.45 μm Nylon Syringe filter	1	98.75	1.78	100.47	1.61
	2	97.93	1.37	97.53	2.33
	3	98.49	0.77	99.52	1.83
0.45 μm PVDF Syringe filter	1	97.36	3.20	98.34	0.53
	2	97.62	1.69	100.08	0.25
	3	97.68	1.59	97.89	0.17

NA = Not applicable

The extraction efficiency results (Table 8) of MTL and CTD Tablets 47.5/12.5 mg (Revelol®–CH 50/12.5) were not affected by changing the sample sonication time from 20 min to 30 min as the results were found within the acceptable range for %relative difference (should be not more than 3.0%).

Table 8: Results of robustness for change in sonication time

Sample sonication (minutes)	Sample no	Metoprolol Succinate			Chlorthalidone		
		%Assay	Average	%RD	%Assay	Average	%RD
25 (As per method)							
	1	99.37	98.99	NA	100.01	99.46	NA
	2	99.47			98.74		
	3	98.11			99.64		
20	1	100.41	99.51	0.52	98.80	98.88	0.59
	2	99.50			97.85		
	3	98.61			99.98		
30	1	98.74	100.69	1.70	100.04	99.38	0.08
	2	102.54			99.67		
	3	100.78			98.43		

NA = not applicable; % RD = % relative difference

Table 9: Robustness results for change in chromatographic parameters

Variation in Chromatographic conditions	Retention time (minute)		Plate count		Tailing factor		%RSD		Reso.	Retention time from sample	
	MTL	CTD	MTL	CTD	MTL	CTD	MTL	CTD		MTL	CTD
As per method	2.033	3.787	2681	7063	1.57	1.34	0.16	0.08	9.56	2.040	3.768
Flow rate – 0.1 mL/min	2.245	4.185	2719	7539	1.58	1.34	0.40	0.52	9.71	2.261	4.185
Flow rate + 0.1 mL/min	1.831	3.449	2498	6917	1.59	1.32	0.67	0.78	9.65	1.868	3.457
Organic phase – 10%	2.257	4.337	2346	7051	1.50	1.33	0.27	0.38	9.85	2.263	4.364
Organic phase + 10%	1.866	3.259	4542	7117	1.56	1.32	0.32	0.31	10.50	1.885	3.277
pH of buffer – 0.1	1.979	3.637	3114	7341	1.59	1.36	0.10	0.24	10.64	1.977	3.648
pH of buffer + 0.1	2.011	3.564	2957	7212	1.57	1.37	0.33	0.32	9.83	1.982	3.588
Quantity of PDP – 10%	1.975	3.589	3415	7576	1.58	1.37	0.19	0.44	10.75	1.966	3.618
Quantity of PDP + 10%	1.964	3.640	3442	6751	1.57	1.27	0.40	0.78	10.77	2.066	3.802
Acceptance criteria	–	–	> 2000		8 – 2.0		<2.0		> 6.0	Similar to standard	

Reso. = USP Resolution between Metoprolol and Chlorthalidone peak

The results of system suitability, system repeatability and change in retention time were checked for each changed method parameter during robustness. Each chromatographic method parameter fulfills the acceptance criteria even after making the deliberate changes into it, indicating its robustness (Table 9).

Solution stability

The results of standard solution stability (Table 10 and Table 11) were meets the acceptance criteria (%relative difference between initial and studied timepoint is NMT 2.0%) revealed that standard solutions were stable for 2 days at bench top (room temperature) and in refrigerator (2-8°C).

The sample solutions were stable for 2 days at bench top (room temperature) and in refrigerator (2-8°C) as the %relative difference between the %assay results obtained (Table 12) from stored sample solutions and initial sample solutions meets the acceptance criteria (the %relative difference between initial and studied timepoint is NMT 3.0%).

The mobile phase appearance was found to be clear and free of visible particles during estimation of mobile phase stability. Also, the system suitability and system repeatability results (Table 13) complies the acceptance criteria, hence the mobile phase was stable for 2 days at bench top (room temperature).

Range

The method range is from 60 to 140% of the nominal working standard solution has been derived for Metoprolol Succinate and Chlorthalidone, based on acceptable linearity, accuracy and precision study results.

Comparison with reported methods for simultaneous estimation of MTL and CTD

After, doing in depth study in present research work in comparison with previously reported work, it was found that the present research work is having various advantages like less time consuming process, short run time and improved resolution. The system suitability and system repeatability results established with two standard solution preparations. Robustness for change in sonication time and filter compatibility study provides additional benefit. The solution stability was reported for mobile phase preparations, standards and samples as well. The forced degradation study also performed on drug substance and drug product with suitable experimental design. Also, it is more economic as require less time of analysis with simple solution preparations. The results obtained from the validation suggest that this method was more simple, rapid, specific, precise, accurate, linear and robust enough as compare to earlier reported methods.

Table 10: First standard solution stability

Time in Days	Room temperature			Refrigerator (2–8°C)		
	Response/mg		%Relative Difference	Response/mg		%Relative Difference
	Fresh standard	Stored standard		Fresh standard	Stored standard	
			Metoprolol Succinate			
Initial	7393.7605	NA	NA	7393.7605	NA	NA
1	7539.9036	7613.4454	0.97	7539.9036	7401.1134	1.88
2	7212.8805	7239.2437	0.36	7212.8805	7217.2269	0.06
			Chlorthalidone			
Initial	18758.1746	NA	NA	18758.1746	NA	NA
1	18231.2195	18585.6349	1.91	18231.2195	18409.4444	0.97
2	18355.4194	18445.1587	0.49	18355.4194	18243.8889	0.61

Table 11: Second standard solution stability

Time in Days	Room temperature			Refrigerator (2–8°C)		
	Response/mg		%Relative Difference	Response/mg		%Relative Difference
	Fresh standard	Stored standard		Fresh standard	Stored standard	
			Metoprolol Succinate			
Initial	7409.3263	NA	NA	7409.3263	NA	NA
1	7539.9036	7630.6105	1.19	7539.9036	7564.0000	0.32
2	7212.8805	7158.5895	0.76	7212.8805	7153.6421	0.83
			Chlorthalidone			
Initial	18742.8800	NA	NA	18742.8800	NA	NA
1	18231.2195	18389.8400	0.86	18231.2195	17912.5600	1.78
2	18355.4194	18700.2400	1.84	18355.4194	18450.8000	0.52

Table 12: Summary results for stability of sample solutions

Time in Day	Sample	Room Temperature				Refrigerator (2 – 8°C)			
		% Assay		%Relative Difference		%Assay		%Relative Difference	
		MTL	CTD	MTL	CTD	MTL	CTD	MTL	CTD
Initial	1	99.37	100.01	NA	NA	99.37	100.01	NA	NA
	2	99.47	98.74	NA	NA	99.47	98.74	NA	NA
	3	98.11	99.64	NA	NA	98.11	99.64	NA	NA
Day 1	1	101.58	101.97	2.20	1.94	100.64	100.21	1.27	0.20
	2	98.23	100.21	1.25	1.47	100.78	100.00	1.31	1.26
	3	98.55	101.56	0.45	1.91	98.90	100.03	0.80	0.39
Day 2	1	101.07	99.18	1.70	0.83	100.73	97.95	1.36	2.08
	2	98.12	101.05	1.37	2.31	97.23	99.02	2.28	0.28
	3	99.37	98.06	1.28	1.60	100.20	99.17	2.11	0.48

NA = not applicable

Table 13: Mobile phase stability results

Time in days	Retention time (minute)		Plate count		Tailing factor		%RSD		Reso.	RT from sample	
	MTL	CTD	MTL	CTD	MTL	CTD	MTL	CTD		MTL	CTD
Initial	1.960	3.626	3587	7526	1.59	1.28	0.29	0.52	11.18	1.978	3.633
1	2.062	3.765	3012	7131	1.57	1.32	0.44	0.49	10.36	1.982	3.610
2	1.996	3.623	3711	6991	1.59	1.34	0.73	0.53	10.69	1.999	3.626
Acceptance criteria			> 2000		0.8 – 2.0		<2.0		> 6.0	Similar to standard	

Reso. = Resolution between MTL and CTD peak; RT = Retention time in minutes

CONCLUSION

Stability indicating RP-HPLC method for simultaneous determination of MTL and CTD in bulk and in tablet dosage form was developed and validated as per guidelines of ICH. Experimental results confirmed that the chromatographic method is linear in the range of studied concentration as well as precise, specific, accurate and robust. The forced degradation study results reveals that all the peaks due to degradant were significantly resolved from the active components peaks, demonstrating the method is stability indicating and specific. The results of %recovery for dosage forms demonstrate that, no interference from the excipients in the determination of active analytes. The values of %RSD were < 2.0 for method repeatability and intermediate precision signifying the high level of method precision. The detection limits and quantitation limits values found to be extremely low, which provides an additional benefit. The robustness results were also demonstrates the working dimension of the method with changes made in different parameters. The solution stability results showed that standard, samples as well as mobile phase solution are stable for 2 days. Also, analysis of drugs is rapid and cost-effective as method has simple isocratic elution with easy extraction as well as sample preparation process. The proposed method can be used for routine analysis and stability studies of MTL and CTD in the quality control of finished product as well as in bulk manufacturing also. However, the separation and structural characterization of degradation products were not carried out.

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Conflicts of interest

The authors declare no conflict of interest.

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