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STABILITY INDICATING HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF RAMIPRIL AND CHLORTHALIDONE IN ITS PHARMACEUTICAL DOSAGE FORM

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Keywords:

Ramipril, Chlorthalidone, HPLC, Stability indicating method

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ABSTRACT

A Simple, economic, precise and Stability indicating RP-HPLC method was developed and validated as per ICH guideline. Chromatographic separation was achieved on Reversed-Phase, Hypersil C18 column (250 × 4.6 mm, 5 µm) using mobile phase consisting of 10mM Dipotassium Hydrogen Phosphate buffer (pH 5.8) and Methanol (40:60, v/v), at a flow rate of 1.5 ml/min. Spectrophotometric UV detection was performed at 215 nm and sharp peaks were obtained at Retention time of 3.509 ± 0.000577 and 6.302 ± 0.001732 minfor Ramipril and Chlorthalidone respectively. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 80-120 µg/mL for Ramipril and 100-150 µg/ mL for Chlorthalidone. Good accuracy and precision were obtained as revealed from %RSD value less than 2. Moreover Different Forced Degradation Studies like Acid and Alkali hydrolysis, Oxidation, Thermal and photo degradation were performed. The utility of the procedure was verified by its application to marketed formulations that were subjectedto accelerated stability studies. The method well separated the drug and degradation products even in actual samples. Extensive Degradation was found to occur in Alkaline medium, and Mild Degradation was observed in Thermal condition for Ramipril and Chlorthalidone.

INTRODUCTION

Ramipril Chemically, (2S,3aS,6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events. Its empirical formula is $C_{23}H_{32}N_2O_5$ and its molecular weight is 416.51g/mol (Figure 1).[1-4]

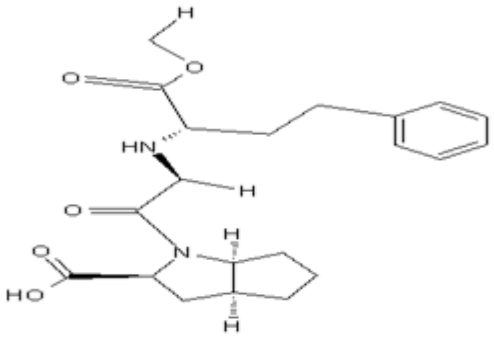

FIG NO.-1: STRUCTURE OF RAMIPRIL

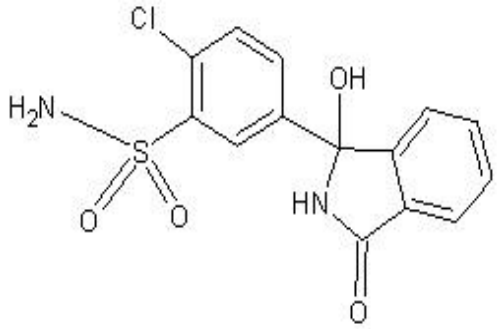

FIG NO. - 2: STRUCTURE OF CHLORTHALIDONE

Chlorthalidone Chemically, 2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide is a thiazide like diuretics. Chlorthalidone is a monosulfonamyl diuretic. Chlorthalidone is used alone or with other hypertensive drug in the management of hypertension and edema. Its empirical formula is $C_{14}H_{11}ClN_2O_4S$ and its molecular weight is 338.8 g/mol (Figure 2). [5-8]

Literature survey reveals that many analytical methods have been reported for determinationof Ramipril and Chlorthalidone individually. No single method was reported for the Stability indicating assay method in combined dosage form. The present work describes the development of a stability indicating RP-HPLC method, which can quantify these components simultaneously from a combined dosage form and also separate this component from its degradation products. The International Conference on Harmonization (ICH) guideline entitled “Stability testing of new drug substances and products” requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. An ideal stability-indicating method is one that resolves the drug and its degradation products efficiently. Consequently, the implementation of an analytical methodology to determine Ramipril and Chlorthalidone simultaneously, in presence of its

degradation products is rather a challenge for pharmaceutical analyst. Therefore, it was thought to study the stability of Ramipril and Chlorthalidone under acidic, alkaline, oxidative, Photo and Thermal conditions. This paper reports validated stability-indicating HPLC method for simultaneous determination of Ramipril and Chlorthalidone in presence of their degradation products. The proposed method is simple, accurate, reproducible, stability indicating and suitable for routine determination of Ramipril and Chlorthalidone in combined dosage form. [9-11]

MATERIALS AND METHODS

Materials and Chemicals

Reference standards of Ramipril and Chlorthalidone were kindly gifted by Ratnamani Pharmaceutical Ltd, Chhatral, Gujarat, India. The Pharmaceutical formulation containing 10 mg Ramipril and 12.5 mg Chlorthalidone (Eris Pharmaceutical Ltd.) was purchased from market. All Solvents and Chemicals used were of analytical grade, purchased from Chemdyes Corporation, Rajkot.

Instrumentation

HPLC (Analytical Technologies Ltd., Vadodara) Model no. S1122 with a rheodyne injector (20 µl fixed loop) and UV Probe UV–visible detector was used. Column- C18 (particle size 5µm; 250mm×4.6mm) was used. Digital pH meter of Systronics and AnalyticalBalance of Swisserwas used.

Preparation of Analytical Solutions

Preparation of Standard stock solutions

Accurately Weigh 100 mg of RAM and 125 mg of CHL was transferred to 100 ml of volumetric flask, dissolved Separately and diluted up to mark with Diluent to give a stock solution (1000µg/ml and 1250 µg/ml)

Preparation of Working Standard Solution

Accurately take 10 ml from above Stock solution and transferred to 100 ml Volumetric flask and Diluted up to mark with Diluent to give a Working std solution of RAM (100 µg/ml) and CHL(125 µg/ml).

Method Development

A variety of mobile phases were investigated in the development of an HPLC method suitable for analysis of RAM and CHL in the bulk drug. These included Water : Methanol, 50:50 (v/v), 0.2 % Orthophosphoric acid in water : Methanol, 50:50 (v/v), 0.2 %

Orthophosphoric acid + 0.2 % Triethylamine in water : Methanol, 50:50 (v/v), 10mM Dipotassium hydrogen Phosphate : Methanol, 50:50 (v/v), and 10mM Dipotassium hydrogen Phosphate : Methanol, 40:60 (v/v) (pH 5.8) The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, suitability for stability studies, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents.

Method Validation

The developed method was validated by determination of various analytical method validation parameters like accuracy, precision, linearity, range, limit of detection, limit of quantitation, robustness, specificity according to ICH guideline Q2(R1) and system suitability according to USP.

Linearity

The linearity was evaluated by linear regression analysis. The calibration graph was plotted for RAM and CHL.

LOD and LOQ

As per ICH guideline, limit of detection and quantitation of the developed method were calculated from the standarddeviation of the response(σ) and slope of the calibration curve(S) of each drug using the formula, Limit of detection=3.3*σ/S; Limit of quantitation=10*σ/S

Precision

Intra-day precision of the method was evaluated for mixtures of RAM and CHL at 3 different concentrations 80:100, 100:125 and 120:150 µg/ml (n=3) by determining their assay.Inter-day precision of the method was tested for 3 days at the same concentration levels. Solutions for calibration curves were prepared fresh every day.

Accuracy

The Standard was spiked with Formulation at these concentration levels of 50, 100, 150% and the mixture were analyzed by the proposed method. The experiment was conducted in triplicate.

Specificity

The specificity of the method was determined by analyzing standard drug and sample.

Robustness

Robustness was evaluated by studying the influence of small deliberate changes of the analytical parameters such as mobile phase, flow rate and pH of Mobile Phase.

System Suitability

System suitability was determined from six replicate injections of the standard solution before the sample analysis. To Check System Suitability Number of Theoretical Plates, Resolution, Retention Time and Tailing Factor were Determine.

Analysis of Marketed Formulation

Twenty Capsuleswere Weight and powdered. An Accurately Weighed powder equivalent to about 50 mg of RAM and 62.5 mg of CHL was transferred to50 mlvolumetricflask, addedabout 35 mlof diluents in to it, sonicatedfor 30minuteswithintermittent shaking, cooledtoattain room temperatureandmadeup tovolumewithdiluent and mixedwell.Itwasfilteredthrough0.45µ syringefilter. 5mlofobtainedfiltratewasfurther diluted to50mlwithdiluent, mixedwell to Prepare 100 µg/ml of Ramipril and 125 µg/ml of Chlorthalidone Solution andit wasinjected.

Force Degradation Study of Ramipril and Chlorthalidone

To evaluate the stability indicating property of the developedHPLC method, RAM and CHL was subjected to forced degradation conditions according to following procedures like acid/ base hydrolysis, oxidation, thermal and photo-degradation.

RESULT AND DISCUSSION

Method Development

The detection was carried out in the UV region and wavelength selected for detection was 215 nm in Methanol. The mobile phase consisting of 10Mm Dipotassium hydrogen phosphate: Methanol (40:60 v/v), pH 5.8 was adjusted with orthophosphoric acid; at 1.5 ml/min flow rate was optimized which gives two sharp well-resolved peaks with minimum tailing factor for Ramipril and Chlorthalidone. The retention times for Ramipril and Chlorthalidone is 3.509 and 6.302 min respectively.

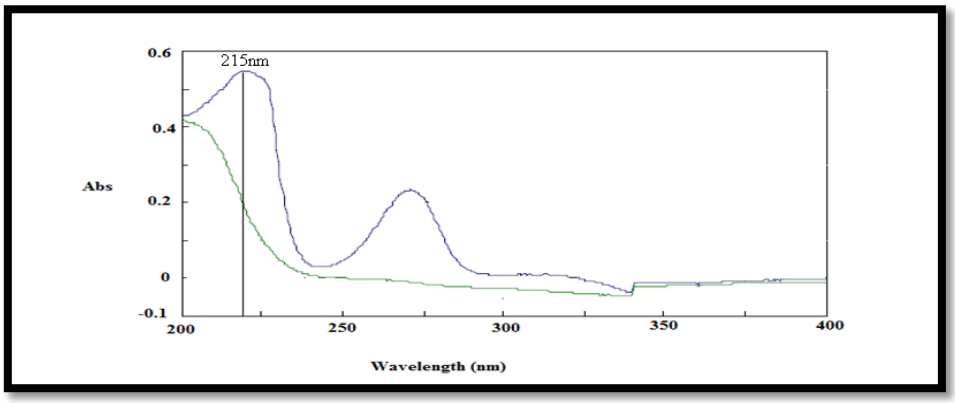

FIG NO.-3: OVERLAY SPECTRA OF RAMIPRIL AND CHLORTHALIDONE

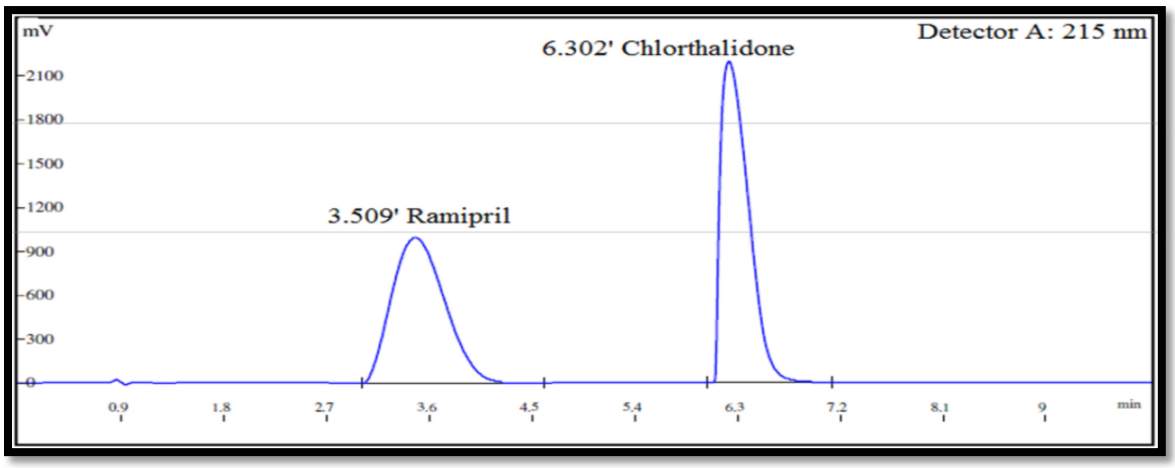

FIG NO. -4: CHROMATOGRAM OF RAMIPRIL AND CHLORTHALIDONE AT OPTIMIZE MOBILE PHASE (10mM DIPOTASSIUM HYDROGEN PHOSPHATE : METHANOL 40 : 60 V/V)

Method Validation

Linearity

The calibration curve for Ramipril and Chlorthalidone were found to be linear over the range of 80-120 µg/ml and 100-150 µg/ml.

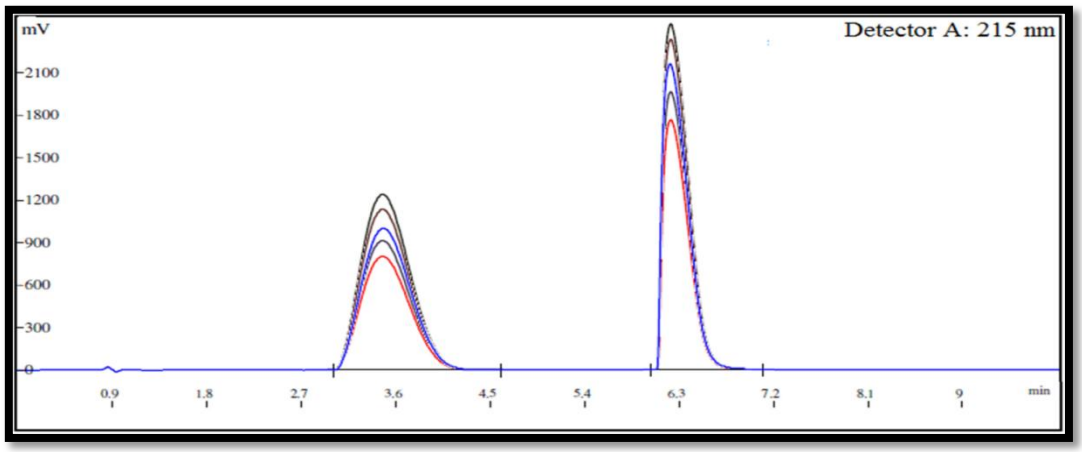

FIG NO. -5: CHROMATOGRAM OF LINEARITY OF RAMIPRIL AND CHLORTHALIDONE

TABLE NO. -1:ANALYTICAL PARAMETERS FOR RAMIPRIL AND CHLORTHALIDONE

Parameters	RAM	CHL
Calibration range (µg/ml)[a]	80-120	100-150
Correlation coefficient (r^2)	0.9913	0.9996
Slope ± S.D	73025 ± 1080.506	69525 ± 4352.468
Confidence interval of slope [c]	58639.02 to 80310.58	43285.58 to 124181.6
Intercept ± S.D	418899 ± 104584.2	163877 ± 198729
Confidence interval of intercept [c]	-260537 to 1809427	-625942 to 2685939
Precision (% RSD)		
Intraday precision	0.10 - 0.32 %	0.18 - 1.17 %
Interday Precision	0.17 - 0.41 %	0.21 - 1.34 %
Accuracy	0.27 - 0.78 %	0.85 - 1.44 %

[a]= 5,c = Confidence interval at 95% confidence level, % RSD = Relative Standard Deviation, S.D = Standard Deviation

Specificity

There was no interference of excipient at the retention time of standard RAM and CHL. So, both drugs can be separated without any interference in sample.

Robustness

The small deliberate variation in HPLC conditions were used to evaluate the robustness of the assay method. The results of analysis of robustness study are as shown in below table, where %RSD less than 2 indicate that the method is robust.

TABLE NO. -2: ROBUSTNESS STUDY OF HPLC METHOD

Parameters	Variation	RAM		CHL	
		Mean[a] ± SD	%RSD	Mean[a] ± SD	%RSD
As Such	-	7810250 ± 43910.66	0.56	8888547 ± 42009.33	0.47
Flow rate (1.5 ml/min)	1.35 ml/min	8574371.17 ± 60869.41	0.70	9762481 ± 30879.5	0.316
	1.65 ml/min	700160.6 ± 27970.07	0.39	8014519 ± 54560.23	0.68
Mobile phase pH (5.8)	pH 5.6	7847182.83 ± 50365.40	0.64	8901317 ± 35144.54	0.39
	pH 6.0	7862291.17 ± 44937.30	0.57	8921866 ± 45793.03	0.51
Mobile phase Ratio (40:60)	38:62	7842895.17± 68186.28	0.86	8902527 ± 41726.59	0.46
	42:58	7852885.17 ± 73696.18	0.93	8829287 ± 60249.09	0.68

n^a= 5, % RSD = Relative Standard Deviation, S.D = Standard Deviation

System Suitability Test

The result of system suitability analysis is shown in below table.

TABLE NO. -3: SYSTEM SUITABILITY PARAMETER OF HPLC

Sr no.	Parameters	Value Obtained		Standard Value
		RAM	CHL	
1.	Retention time (min) (R_t)	3.509	6.302	-
2.	Peak Area	7798160	8772614	% RSD was NMT 2.00
3.	Mean Theoretical plates (N) or Column efficiency	7865	13144	NLT 2000
4.	Mean Tailing factor (A_s) or Symmetry factor	1.1	1.2	NMT 2.00

Analysis of marketed formulation

Analysis of Capsule dosage containing 10 mg of RAM and 12.5 mg of CHL was carried out and the amount recovered were expressed as percentage amount of the label claims. Percentage amount found for all the drugs were within the range of 95% to 105%w/w.

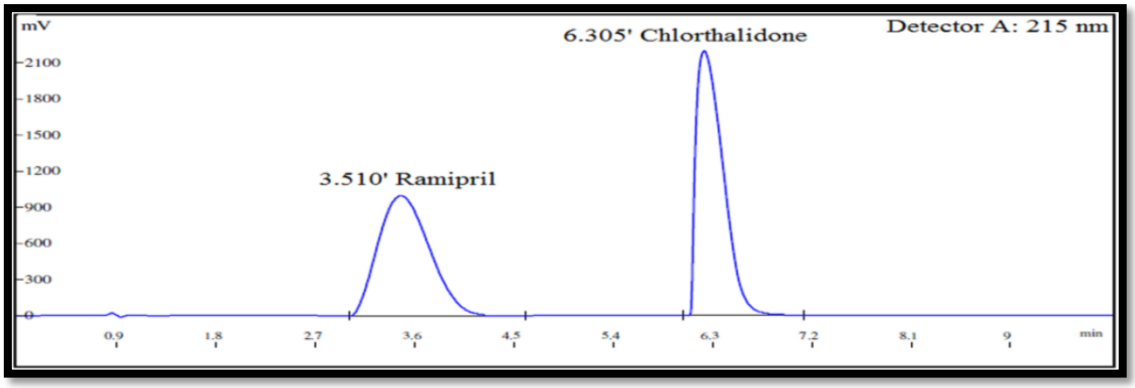

FIG NO. -6: CHROMATOGRAM OF MARKETED FORMULATION (RAMISAVE)

TABLE NO. -4: ANALYSIS OF MARKETED FORMULATION

Drugs	Label claim (mg)	% Amount of drug found	% RSD
RAM	10 mg	100.4 %	0.25
CHL	12.5 mg	99.6 %	0.10

Force Degradation Study

To study the effect of acid, 5 ml of 0.5 M HCl was added to the sample and the mixture was kept for 3 hours. To study the effect of base, 5 ml of 0.5 M NaOH was added to the sample and the mixture was kept for 3 hours. To study the effect of oxidizing conditions, 5 ml of 3% v/v H_2O_2 was added to the sample and the mixture was kept for 3 hours. To study the effect of temperature sample was kept in an oven at 80ºC. To study the effect of sun light was kept in a day light for 12 hours.

TABLE NO. -5: SUMMARY OF FORCED DEGRADATION STUDY

Stress Type	Stress Condition	Ramipril		Chlorthalidone	
		%Assay	%Degradation	%Assay	%Degradation
Control Sample	As Such Sample	100.4%	NA	99.6%	NA
Acid Degradation	2N HCl, 5 ml for 2 hours	85.8%	14.6%	86.7%	12.9%
Base Degradation	2 N NaOH, 5 ml for 3 hours	84.5%	15.9%	86.2%	13.4%
Peroxide Degradation	5 ml 3 % H_2O_2 at RT for 3 hours	89.3%	11.1%	89.5%	10.1%
Thermal Degradation	At80℃for3hours	90.1%	10.3%	91.3%	8.3%
Photo Degradation	At sunlight for 12 hours	89.9%	10.5%	90.1%	9.5%

CONCLUSION

The proposed HPLC method for the simultaneous estimation of RAM and CHL in bulk and combined pharmaceutical dosage forms is accurate, precise, linear, robust, simple, rapid, and selective. It can be adopted efficiently and easily for routine quality control (QC) analysis of raw materials, formulations. Stability indicating assay method can be successfully applied to perform long-term and accelerated stability studies of bulk and combined pharmaceutical dosage formulations of RAM and CHL. It can also be used to check quality of product after different storage condition and when stress degradation is carried out.

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