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Case Study

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF CHLORTHALIDONE AND METOPROLOL SUCCINATEIN BULK AND FORMULATION BY RP-HPLC METHOD

Dr. G.Nagaraju[1*], V. Sirisha[2], Kavati Ramakrishna[3], Dr. Hareesh Dara[1]

[1*] Department of Pharmaceutical Chemistry, Dhanvanthari Institute of Pharmaceutical Sciences, Sujathanagar, Kothagudem.
[1] Department of Pharmaceutics, Sree college of pharmay, nayakulagudem, Kothagudem,Telangana.
[2] Department of Pharmaceutics, Dhanvanthari Institute of Pharmaceutical Sciences, Sujathanagar, Kothagudem.
[3]Department of Pharmaceutics, Pulipati Prasad college of pharmacy, Pulipati Prasad college of pharmaceutical sciences, khammam.

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ABSTRACT

A simple, rapid, precise, accurate, economical and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of Chlorthalidone and Metoprolol Succinate in combined pharmaceutical formulations. The separation was achieved on a Thermo Scientific BDS column (250 × 4.6 mm i. d, particle size of 5 µ) using a mobile phase consisting of Acetonitrile: 0.1% Trimethylamine (90:10 %v/v) (pH adjusted to 4.5with 0.1% orthophosphoric acid). The mobile phase was pumped at a flow rate of 1ml/min. The UV detection was monitored at 224nm. The retention times of Chlorthalidone and Metoprolol Succinate were found to be 2.6min and 6.5min respectively. Excellent linearity range was found between 4-14µg/ml for Chlorthalidone and 16-56µg/ml for Metoprolol Succinate. The method was validated with respect to linearity, precision, accuracy, specificity and ruggedness. Method was successfully applied for the simultaneous determination of Chlorthalidone and Metoprolol Succinate in combined pharmaceutical dosage form.

Keywords:*Chlorthalidone, Metoprolol Succinate, RP-HPLC method*

INTRODUCTION

Chlorthalidone (CLR) is chemically 2-chloro-5-(1-hydroxy-3-oxo-2, 3 dihydro1Hisoindol-1-yl)benzene-1-sulfon-amide (Molecular Formula C14H11CIN2O4S) a monosulfonamyl diuretic, differsfrom other thiazide diuretics in that a double ring system is incorporated into its structure Chlorthalidone is used alone or with atenolol in the management of hypertension and edema[1-3]. Chlorthalidone inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. [4-6] By increasing the delivery of sodiumto the distal renal tubule, Chlorthalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism [7-9].

***Corresponding author:**
Dr. G. Nagaraju
Department of Pharmaceutical Chemistry, Dhanvanthari Institute of Pharmaceutical Sciences, Sujathanagar, Kothagudem.
Email: gdp413@gmail.com
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Metoprolol Succinate is soluble in acetonitrile and methanol.

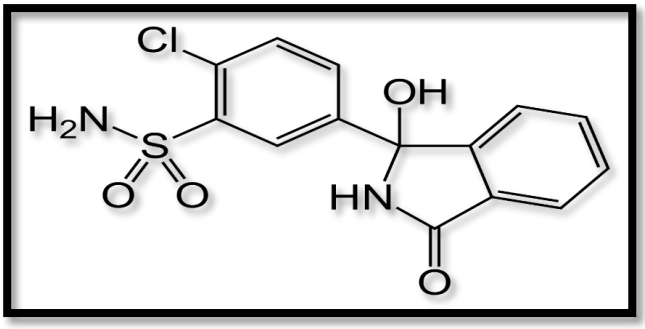

Figure1: Structure of Chlorthalidone

Metoprolol is chemically 1-(isopropyl amino)- 3- [p-(2-methoxyethyl) phenoxy] - 2-propanolSuccinate. (Molecular Formula C15H25NO3) a competitive, beta1-selective (cardioselective) adrenergic antagonist, is similar to atenolol in its moderate lipid solubility, lack of intrinsic sympathomimetic activity (ISA), and weak membrane stabilizing activity (MSA). Metoprololcompetes with adrenergic neurotransmitters such as

catecholamines for binding at beta (1)-adrenergic receptor in the heart. Beta (1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure. It is soluble in water, ethanol and methanol[10].

For this combinationliterature survey revealed that there are a very few methods available for individual andsimultaneous estimation of Chlorthalidone and Metoprolol Succinate. The present work aims atdeveloping a simple, sensitive, accurate and precise method for the effective quantitativeestimation of Chlorthalidone and Metoprolol Succinate as active Pharmaceutical Ingredient (API)as well as in pharmaceutical preparations without the interference of other constituents in thepreparation[11, 12].

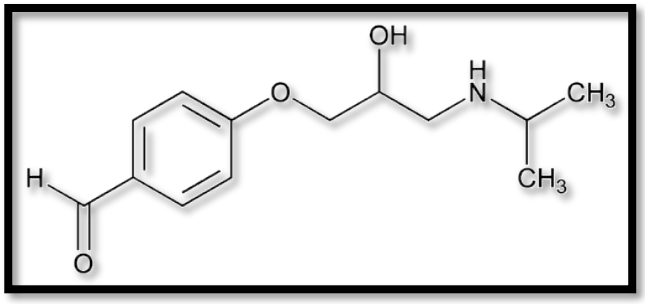

Figure 2: Structure of Metoprolol Succinate

EXPERIMENTAL

Reagents and Chemicals

The drug samples of Chlorthalidone and Metoprolol Succinate were brought from Aurobindo pharma Pvt Ltd. Chlorthalidone (12.5mg) and Metoprolol Succinate (50 mg) were contained in our nearby drug shop. Acetonitrile, Methanol, Orthophosphoric acid, and water are of HPLC grade were supplied by Akshaya Chemicals Ltd., India; and Merck specialties private limited, Mumbai.

Instrumentation and Chromatographic Conditions

Shimadzu LC20AD attached to PDA detector, which is having a Rheodyne injector and autosampler opted for chromatography. A degasser to remove the dissolved air and column oven to maintain the desired temperature is also available in the system. Operation data acquisition and analysis were performed by using LC Solution software.

The mobile phase Acetonitrile and 0.1%tri ethylamine (Tri ethylamine pH4.5, adjusted with orthophosphoric acid) in the ratio of (90:10 % v/v), as mobile phase in an isocratic elution mode, at a flow rate of 1.0 ml/min Stationary phase is Thermo Scientific BDS C18 column (250 × 4.6 mm int. diam 5µ). A Flow rate of the mobile phase was 1.0 ml min-1 and all chromatographicexperiments were performed at room temperature (25 ºC ± 2 ºC). The detector wavelength was fixed at 224 nm.

Preparation of Standard Solution

Stock solutions were prepared by dissolving Chlorthalidone and Metoprolol Succinate in 100 ml of mobile phase separately. Aliquots of the standard stock solutions of Chlorthalidone and Metoprolol Succinate were transferred into 10 ml volumetric flasks and the solution was made up to the volume to yield required concentrations of both drugs within the linearity range.

Preparation of Sample Solution.

Twenty(VINCOR-D) tablets each containing 12.5 mg Chlorthalidone and 50 mg of Metoprolol Succinate. A quantity equivalent to 12.5 mg Chlorthalidone and 50 mg of Metoprolol Succinatewas weighed and transferred into a 250 ml volumetric flask. It is extracted with the mobile phase. The volumetric flask was sonicated for 20 minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with mobile phase and filtered. Suitable aliquots of formulation solution were prepared and injected into HPLC to obtain a concentration in the linearity range

Preparation of Calibration Curve Standards

Linearity was established by least-squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 4-14µg/ml for Chlorthalidone and 16-56µg/ml for Metoprolol Succinate, Fig no. 4 and 5. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. The slope, intercept and the correlation coefficient were found to be 98463, 12842, and 0.998 respectively for Chlorthalidone and 41916, 65446 and 0.997 respectively for Metoprolol Succinate.

Table 1: Calibration data of Chlorthalidone by RP-HPLC

S.No	Concentration(µg/ml)	Retention time (mins)	Peak area
1	4	2.6	380405
2	6	2.6	559434
3	8	2.6	797058
4	10	2.6	969174
5	12	2.6	1184414
6	14	2.6	1349479

Table 2: Calibration data of Metoprolol by RP-HPLC

S.No	Concentration(µg/ml)	Retention time(mins)	Peak area
1	16	6.4	635670
2	24	6.5	892578
3	32	6.5	1300150
4	40	6.5	1602689
5	48	6.5	1930138
6	56	6.5	2299911

Table 3: Linearity

Parameters	Chlorthalidone	Metoprolol
Linearity range	4-14 µg/ml	16-56 µg/ml
Correlation coefficient	0.998	0.997
Slope	98463	41916
Intercept	12842	65446

RESULTS AND DISCUSSION

Method development

Optimal chromatographic conditions were determined after studying various parameters affecting the chromatographic separation of a mixture including the buffer concentration, PH, and flow rate to achieve maximal separation of the drugs and better peak shape. Various combinations of organic solvents in different ratios were tried to obtain a well-resolved chromatogram ofChlorthalidone and Metoprolol Succinate. The concentration and proportion of buffer were varied in the mobile phase to obtain a good peak shape. After the selection of the drug combination, both the drugs were dissolved in a suitable diluent to get a clear solution. Based on the literature reverse-phase chromatography was identified as an appropriate chromatography separation method. The mobile phase was optimized by modifying different combinations of buffers and organic solvents. The pKa values of both the drugs were considered for optimization of the pH of the buffer. The resolution and the peak shape of both the drugs were found significant with the mobile phase composition of Acetonitrile and 0.1%tri ethylamine (Tri ethylaminepH4.5, adjusted with orthophosphoric acid) in the ratio of (90:10 % v/v), at a flow rate of 1mL/min and analyzed at 224 nm. The retention time observed (2.6 min forChlorthalidoneand 6.4 min for Metoprolol Succinate) allows a rapid determination of these drugs. A typical chromatogram is shown below in Figure-3.

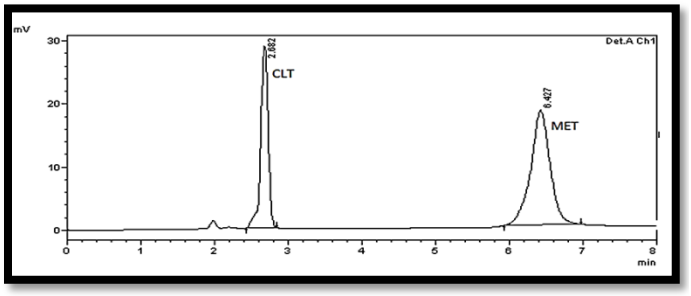

Figure 4: Optimized Chromatogram

METHOD VALIDATION

System suitability studies

System suitability parameters like Retention time, number of theoretical plates (N), Tailing factor, resolution (Rs), etc., were studied, and results are given in Table 4.

Table 4: System Suitability Studies

Drug	Theoretical plates (N)	Retention time(Rt)	Tailing factor	Resolution
CLT	4265	2.6	0.9	3.8
MET	3157	6.4	0.9	

Linearity and Range

Linearity was established by least-squares linear regression analysis of the calibration curve. 4-14µg/ml for Chlorthalidone and 16-56µg/ml for Metoprolol Succinate, Table 1, Fig.4 and Table 2, Fig.5respectively.Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. The slope, intercept and the correlation coefficient were found to be 98463, 12842, and 0.998 respectively for Chlorthalidone and 41916, 65446 and 0.997 respectively for Metoprolol Succinate

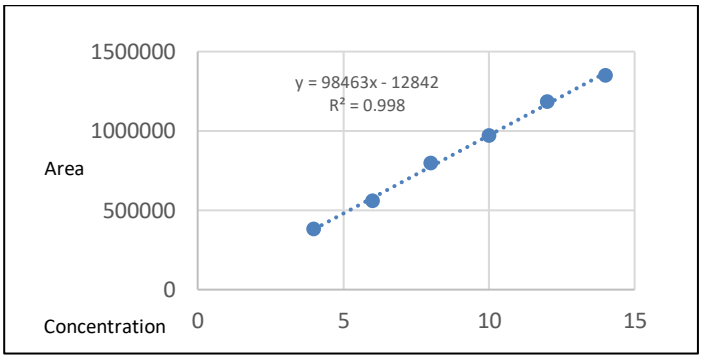

Figure 4: Calibration Graph of Chlorthalidone

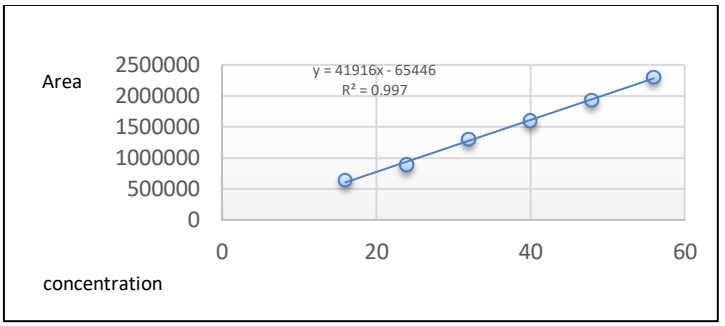

Figure 5: Calibration Graph of Metoprolol Succinate

Precision

Intraday precision

Intraday precision was done by carrying out analysis of standard drug solutions at two different concentrations in the linearity range for three times on the same day and %RSD was calculated, Table 5.

Table 5: Intraday precision

Drug	Concentration(µg/ml)	Peak Area (Avg*)	% RSD
Chlorthalidone	10	965377	0.95
	12	1187943	0.45
Metoprolol	40	1612954	1.16
	48	1927234	0.32

* Mean of six observations

Inter day precision

Inter-day precision was done by carrying out the analysis of standard drug solutions at two different concentrations in the linearity range for three days a period of one week and %RSD was calculated Table 6.

Table 6: Inter day precision

Drug	Concentration (µg/ml)	Peak Area (Avg*)	%RSD
Chlorthalidone	10	965815	0.58
	12	1187973	0.33
Metoprolol	40	1598801	0.34
	48	1924758	0.26

* Mean of six observations

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated mathematically. The LOD of Chlorthalidone andMetoprololSuccinate were found to be 0.61 µg/ml and 2.63 µg/ml respectively. The LOQ of Chlorthalidone andMetoprolol were found to be 1.86µg/ml and 7.98 µg/ml respectively.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate changes in the method parameters and provides an indication of its reliability during normal usage. The robustness of the method was investigated under a variety of conditions including changes inthe composition of buffer in the mobile phase and flow rate. % RSD of the assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

Ruggedness

The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for Chlorthalidone andMetoprolol that has been performed by two analysts. The % RSD values for assays performed in the same laboratory by two analysts did not exceed 2, indicating the ruggedness of the method.

Accuracy

Recovery studies of the drug were carried out for determining accuracy parameters. It was done by mixing a known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out at 50 and 100% levels.

Table 7: Accuracy

Drug	%Recovery		%RSD	
	50%level	100% level	50% level	100% level
CLT	106.2	101.0	0.23	0.49
MET	107.2	101.2	0.35	0.30

Results of recovery are shown in Table 7.

*Mean of three observations

ANALYSIS OF FORMULATION

Fixed chromatographic conditions were made use for the analysis of formulation.

Preparation of standard solutions

Stock solutions containing concentrations of 1000µg/ml of Chlorthalidone and 1000µg/ml Metoprolol Succinate were prepared by using mobile phase. This solution was suitably diluted to get aliquots of standard solutions containing 4-14µg/ml of Chlorthalidone and 16-56 µg/ml of Metoprolol Succinate. Fig.4 and Fig 5.

Preparation of sample solutions

Five tablets (VINCOR-D) are emptied, the contents weighed and the average weight was calculated. A quantity equivalent to 40mg of drug was dissolved in the mobile phase and such that sample contains 12.5 mg of Chlorthalidone and 50 mg of Metoprolol Succinate. Fig no.6

Recording of chromatograms

A steady baseline was recorded with the fixed chromatographic conditions and standard drug solutions were injected and chromatograms were recorded. Retention times of Chlorthalidoneand Metoprolol Succinate were found to be 2.6 and 6.5 minutes. This was followed by injection of sample

solution obtained from the formulation, Fig 6. Calibration curves were plotted using peak areas of standard drug vs. concentration of corresponding standard solutions. Peak areas of the sample chromatograms were compared and the amount of Chlorthalidone and Metoprolol Succinate were calculated, results are given in Table 8.

Fixed chromatographic conditions were made use for the analysis of formulation.

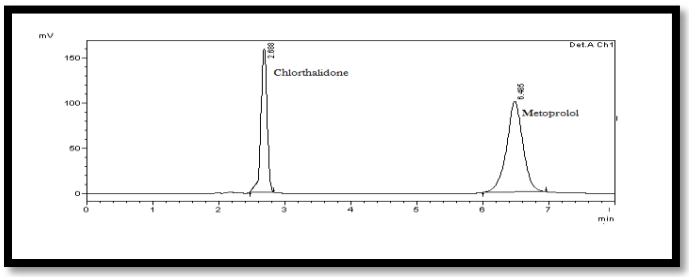

Figure 6: Chromatogram of Formulation

Table 8: Analysis of Marketed Formulation

Drug	Amount(mg/tablets)		%Label claim	%RSD*
	Labeled	Estimated		
CHLOR	12.5	12.27	98.16	0.77
MET	50	49.68	99.36	0.52

* mean of three observations

CONCLUSION

For routine analysis purposes, it is always necessary to establish a method capable of analyzing a huge number of samples in a short time with due accuracy and precision very few analytical methods appeared in the literature for the determination of Chlorthalidone and Metoprolol Succinate by RP-HPLC. In this work,an attempt was made to develop and validate RP-HPLC methods for the estimation of Chlorthalidone and Metoprolol Succinate.

The results expressed in Table 1 to 8for the RP-HPLC method are promising and the proposed methods confirm the suitability of these methods for pharmaceutical dosage forms. The excipients usually present in the pharmaceutical formulation did not interfere with the estimation when some marketed formulations were analyzed by these methods. The accuracy of the methods was confirmed by recovery studies by adding known amounts of pure drugs to the formulation already analyzed by this method.These methods can be used for the routine determination of Chlorthalidone and Metoprolol Succinate in bulk and pharmaceutical dosage forms.

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