METHOD VALIDATION AND DEVELOPMENT OF CHLORTHALIDONE BY RP-HPLC

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ABSTRACT : The objective of the present research work was to develop a innovative, simple, and economic method for estimation of Chlorthalidone in bulk and dosage form by RP-HPLC.The chromatographic conditions were performed on Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d.as stationary phase and mobile phase was prepared with a mixture of Phosphate dihydrogen phosphate buffer : Methanol = 55:45 (pH-3.4) flow 1.0 ml/min, with Injection Volume 20µl, at detection wavelength 244 nm and run time at 7.0 min.The analytical method is valid for estimation of Chlorthalidone over a range of 6µg/ml–14 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range.A specific, sensitive, economic method estimation of Chlorthalidone has been developed based on ICH Guidelines with bulk and dosage forms.
Key Words: Chlorthalidone, HPLC, Method Development, ICH, Validation, Accuracy, Precision.

I.INTRODUCTION

Chlorthalidone (CHL) is oral diuretic oral antihypertensive agent which is chemically described as (RS) 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide (figure 1a) [1]

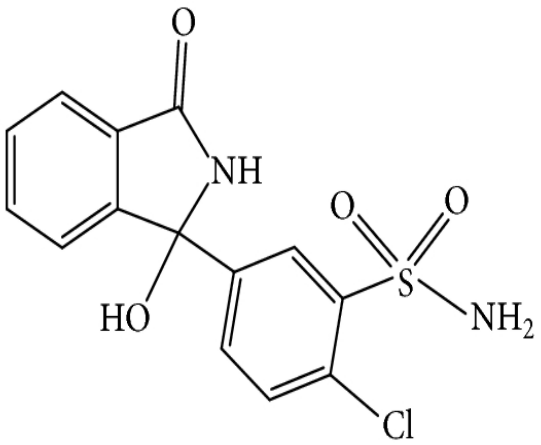


Fig-1: Structure of Chlorthalidone

Literature survey revealed that CHL can be estimated by spectrophotometry[2], RP-HPLC[3-5] and by HPTLC[6] individually and in combination with other drugs. Several methods have been described for the determination of IBS by UV Spectrophotometry[8,9], HPLC[10,11], UPLC[12] and HPTLC[13] individually and in combination with other drugs.

II.EXPERIMENTAL

2.1 Materials and Methods:

Pharmaceutical grade working standard Chlorthalidone were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system

EMPOWER2 software, UV-Visible double beam spectrophotometer (ELICO SL-159), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Sample & Standard Preparation for the Analysis

25 mg of Chlorthalidone standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

2.4 Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Chlorthalidone solution we observed the maxima at 244 nm.

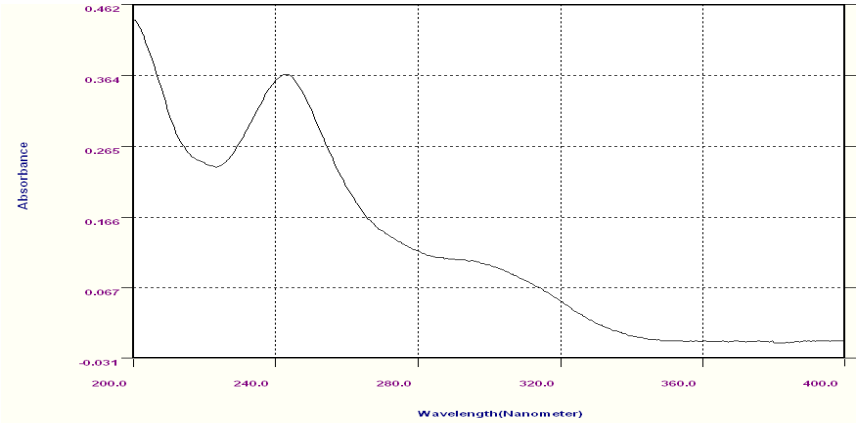


Fig-2: UV Spectrum for Chlorthalidone

2.5 Method Development

2.5.1 Preparation of Phosphate buffer (pH-3.4) Solution:

About 6.80 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 3.4 with diluted orthophosphoric acid.

2.5.2 Preparation of Mobile Phase:

The mobile phase used in this analysis consists of a mixture of Pot.di hydrogen phosphate buffer (pH adjusted to 3.4 with ortho phosphoric acid) and Methanol in a ratio of 55:45. 550mL (55%) of Pot.di hydrogen Phosphate Buffer (pH adjusted to 3.4 with ortho phosphoric acid) and 450mL of Methanol (45%) of above prepared phosphate buffer were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

2.5.3 Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimised Chromatographic Conditions

Mobile phase	Pot.dihydrogen Phosphate Buffer: Methanol= 55:45 (pH-3.4)
Column	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.
Flow rate	1.0 ml/ min.
Wavelength	244nm
Sampling System	Automatic
Temp. of Auto sampler	Ambient
Volume of injection	20µl
Run time	07min.
Mode of Separation	Isocratic

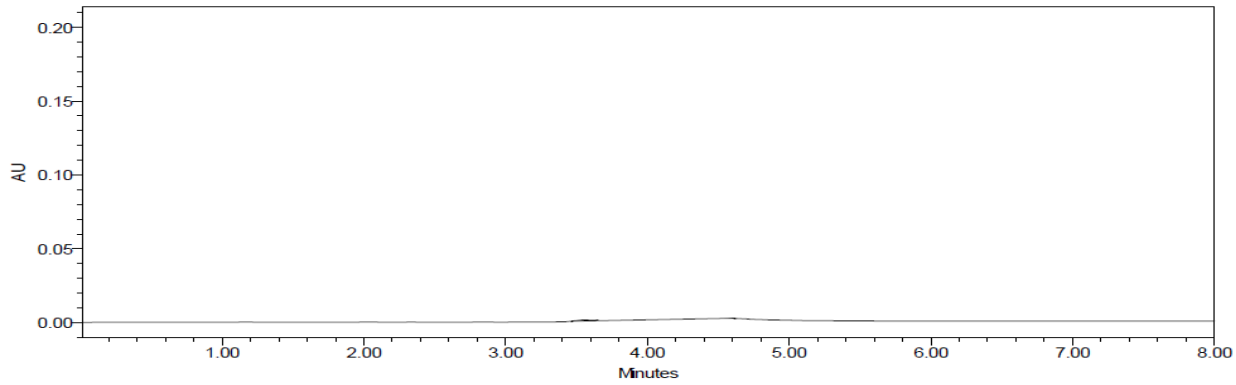
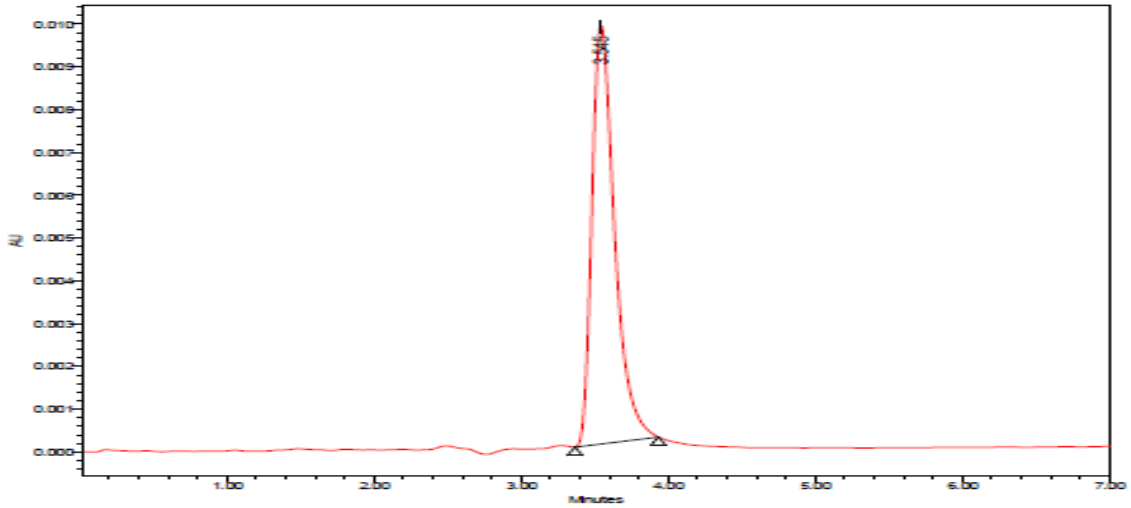


Fig-3: Chromatogram for Blank Preparation



Fig–4: Chromatogram of Chlorthalidone in Optimized Condition

2.6 Method validation:

2.6.1 Linearity & Range:

Calibration standards at five levels were prepared by appropriately mixed and further diluted standard stock solutions in the concentration ranges from 6-14µg/mL for Chlorthalidone. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs. Chromatograms of each solution were recorded.

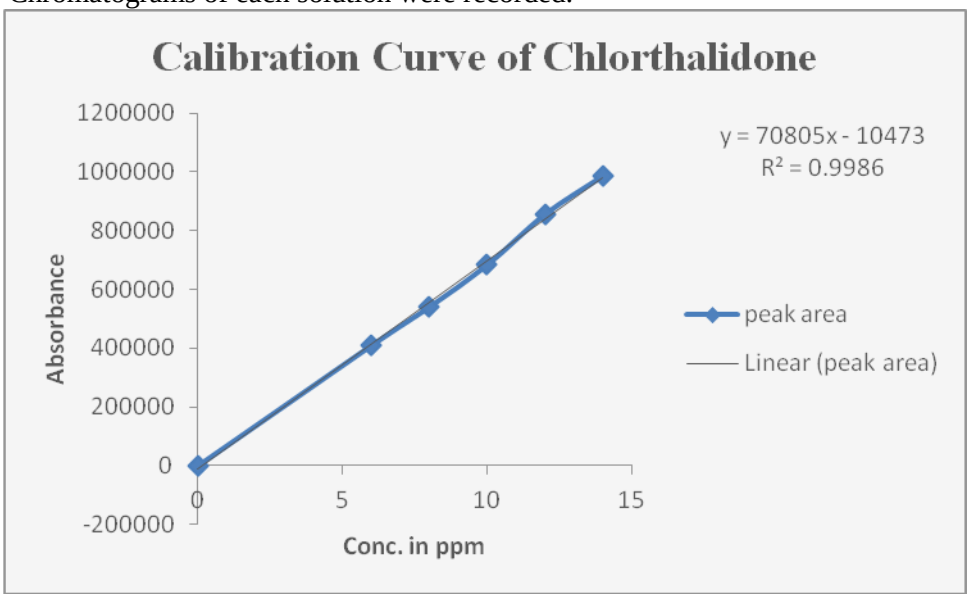


Fig-5: Calibration Curve of Chlorthalidonre (API)

Table-2: Concentration of Chlorthalidone

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
6	409852
8	540864
10	684126
12	856125
14	986458

2.6.2. Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Chlorthalidone were taken and added to the pre-analyzed formulation of concentration 100µg/ml. From that percentage recovery values were calculated. The results were shown in table-3.

Table-3: Accuracy Readings of Chlorthalidone

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	7.9	569989	98.77763	Mean= 99.1357% S.D. = 1.056284 % R.S.D.= 1.06
S_2 : 80 %	8	8.0	578751	100.3245	
S_3 : 80 %	8	7.8	567312	98.30503	
S_4 : 100 %	10	9.8	709788	98.76633	Mean= 100.1784% S.D. = 1.238596 % R.S.D.= 1.2385
S_5 : 100 %	10	10.0	723395	100.6881	
S_6 : 100 %	10	10.1	726176	101.0809	
S_7 : 120 %	12	11.8	846927	98.44573	Mean= 99.57136% S.D. = 1.30981 % R.S.D. = 1.315
S_8 : 120 %	12	11.9	853840	99.25935	
S_9 : 120 %	12	12.1	868706	101.009	

2.6.3. Precision:

2.6.3.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug.Chlorthalidone (API). The percent relative standard deviation was calculated for Chlorthalidone are presented in the Table-4.

Table-4: Repeatability Readings of Chlorthalidone

HPLC Injection Replicates of Chlorthalidone	Retention Time	Peak Area
Replicate – 1	3.538	704122
Replicate – 2	3.540	704232
Replicate – 3	3.537	702658
Replicate – 4	3.545	702541
Replicate – 5	3.547	702863
Replicate – 6	3.545	702754
Average	**3.542**	**703195**
Standard Deviation	**0.004195**	**768.8287**
% RSD	**0.118443**	**0.10933**

2.6.3.2. Intermediate precision:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Chlorthalidone revealed that the proposed method is precise.

Table-5: Results of Intra-Assay & Inter-Assay

Conc. Of Chlorthalidone (API) (µg/ml)	Observed Conc. Of Chlorthalidone (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.79	0.61	8.10	0.95
10	9.52	0.50	10.11	0.92
12	12.01	0.65	11.92	0.72

2.6.4. Method Robustness:

Influence of little changes in optimized chromatographic conditions like changes in flow rate (± 0.1ml/min), mobile phase ratio (±2%), Wavelength of detection (±2nm) and Acetonitrile content in mobile phase (±2%) studied to measure the robustness of the method are also in favour of (Table-36, % RSD < 2%) the developed RP-HPLC method for the analysis of Chlorthalidone (API).

Table-6: Results of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.64
Flow (0.9 ml/min)	0.68
More Organic	0.71
Less Organic	0.68
Wavelength of Detection (246 nm)	0.94
Wavelength of detection (242 nm)	0.92

2.6.5. LOD & LOQ:

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

2.6.6. System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-7.

Table-7: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	8.4
2	Asymmetry	T ≤ 2	Chlorthalidone =0.19
3	Theoretical plate	N > 2000	Chlorthalidone =3245
4	Tailing Factor	T<2	Chlorthalidone =1.09

2.6.7 Estimation of Chlorthalidone in Tablet Dosage Form

Twenty tablets were taken and the I.P. method was followed to calculate the average weight. Above weighed

tablets were finally powdered and triturated well. Some quantity of powder which is equivalent to 25 mg of drug was transferred to a clean and dry 25 ml volumetric flask, make and solution was sonicated for fifteen minutes. Then the volume was made up to 25 ml with the same Mobile Phase. Then 10 ml of the prepared above solution was diluted to 100 ml with the help of mobile phase. The resulted solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The final solution prepared was injected in 5 replicates into the HPLC system and the s are record the observations. Two duplicate injections of the standard solution were also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-8.

Table-8: Assay of CHLORTHALIDONE Tablets

Brand name of Chlorthalidone	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Thalitone (15 mg) (Monarch pharmaceuticals Inc.)	15mg	14.86 (± 0.488)	99.06 (± 0.353)

III.RESULTS

The optimized chromatographic conditions were Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d.as stationary phase and mobile phase was prepared with a mixture of Pot.di hydrogen Phosphate Buffer: Methanol = 55:45 (pH-3.4) flow 1.0 ml/min, with Injection Volume 20µl, at detection wavelength 244 nm and run time at 7.0 min. In these chromatographic conditions the peak was pure, sharp, symmetric and found a greater number of theoretical plates.

The results obtained in method validation were

Linearity & Range: The calibration curve showed good linearity in the range of 6-14 µg/ml, for Chlorthalidone (API) with correlation coefficient (r^2) of 0.999 (Fig-5). A typical calibration curve has the regression equation of y = 70805x + 10473 for Chlorthalidone.

Accuracy: The mean recoveries were found to be 98.9595, 100.7166 and 100.40737 for Chlorthalidone. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Repeatability: The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml for Chlorthalidone (n =6) showed %RSD of **0.10933**%. It was concluded that the analytical technique showed good repeatability.

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.27 µg/ml respectively.

Assay: The assay in Thalitone tablets containing Chlorthalidone was found to be 99.06 %.

IV.DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Chlorthalidone, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.

Vilas Sawale*[14] et al., Objective: To develop and validate a simple, rapid, accurate and precise RP-HPLC method for the simultaneous determination of olmesartan medoxomil (OLM) and chlorthalidone (CHL) in pharmaceutical formulation. Methods: Chromatographic separation was performed on a Phenomenox, Gemini C18 (250×4.6 mm, 5 µm) column from thermo isocratic mode with mobile phase 55:45 water: acetonitrile with pH adjusted to 3.0 with ortho phosphoric acid at flow rate 1 ml/min. Peak intensity of both the drugs was monitored at 250 nm with UV detection. Results: The retention time (RT) of OLM and CHL was found to be 2.95 and 3.91 min, respectively. **S. S. Aher*[15] et al.,**Objective: A simple, precise, accurate method was developed for the simultaneous estimation of azilsartan and chlorthalidone in bulk and tablet dosage form by RP-HPLC technique. Methods: Acetonitrile and water in the ratio of (70:30) pH 2.8 used as mobile phase run through

(Cosmosil C18 (4.6ID x 250 mm, Particle size: 5 micron) column with a flow rate of 0.9 ml/min. The temperature of the column oven was maintained at 30 °C. Wavelength was selected 244 nm. Stock and working solutions were prepared by using the diluents water and acetonitrile in the ratio of 50:50. Runtime was fixed to 9 min **G. S Kumar*[16] et al.,**A reverse phase high performance liquid chromatography (RP HPLC) method was developed and validated for the simultaneous estimation of atenolol and chlorthalidone in marketed formulation. The determination was carried out on an Xterra RP8 (150 x 4.6 mm, 5 µm) column using a mobile phase of potassium dihydrogen phosphate buffer solution: methanol (50:50v/v, pH 3.6) with flow rate 0.5ml/min (UVdetection at 240 nm). The retention time for atenolol was 3.2 min and for chlorthalidone 5.0 min. **Gita Chawla*[17] et al., Objective:** Development of an accurate, precise, robust, sensitive, economical and rapid isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) method complying quality by design (QbD) trends for simultaneous estimation of azilsartan medoximil and chlorthalidone in bulk and formulation form and validation of the method as per ICH guidelines. Methods: The simultaneous estimation of the drugs-azilsartan and chlorthalidone was performed using C8 column having dimensions 150×4.6 mm×5 µm, injection volume 10 µl, flow rate 0.8 ml/min., runtime 10 min., column temperature 20 o Results: The retention times for chlorthalidone and azilsartan medoxomil were 2.4 min. and 5.1 min. respectively with resolution 17. **Lalitha K G*[18] et al.,**A Simple precise and accurate method was developed and validated for the simultaneous analysis of chlorthalidone and irbesarton in tablet formulations. The method has been shown adequate separation of the two ingredients from each other. The chromatographic separation was achieved on a reverse phase column C18 (250 mm x 4.6 mm, 5µ), in a mobile phase consisting of 0.02 M ammonium phosphate buffer (adjusted to pH 5.5 with triethyl amine), acetonitrile and methanol in the ratio (40:40:20, v/v/v) at a flow rate of 1 ml/min with UV detection at 220 nm.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Chlorthalidone in formulations.

V.CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Chlorthalidone API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Chlorthalidone indicated that the developed method is specific for the estimation of Chlorthalidone. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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