STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION CHLORTHALIDONE IN API AND TABLET DOSAGE FORM

*NIHARIKA MUTHYALA, B. NARESH**
Pharmaceutical Analysis and Quality Assurance, Talla Padmavathi Pharmacy College, Urus, Kareembad, Warangal, India.

ABSTRACT *A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of Chlorthalidone in bulk and pharmaceutical dosage formdosage form. The chromatographic method was standardized using Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column with UV detection at 245 nm and 0.1% Orthophosphoric acid: Acetonitrile: Methanol (12:18:70 v/v/v) ratio at a flow rate of 1.0 ml/ min.The proposed method was successfully applied to the determination of Chlorthalidone in bulk and pharmaceutical dosage form. The method was linear over the range of 0-14µg/ml. The recovery was in the range of 98% to 102% and limit of detection was found to be 0.08 µg/ml and quantification was found to be 0.24 µg/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines.*

Keywords:RP-HPLC, Chlorthalidone, Method development and validation,ICH Guidelines.

I.INTRODUCTION

Chlortalidone, also known as chlorthalidone, is a diuretic medication used to treat high blood pressure, swelling including that due to heart failure, liver failure, and nephrotic syndrome, diabetes insipidus, and renal tubular acidosis.[1-4] In high blood pressure it is a preferred initial treatment; in resistant high blood pressure chlortalidone is preferred over hydrochlorothiazide. It is also used to prevent calcium-based kidney stones.[5-7] It is taken by mouth. Effects generally begin within three hours and last for up to 3 days.Common side effects include low blood potassium, high blood sugar, dizziness, and erectile dysfunction.[8-10] Other serious side effects may include gout, allergic reactions, and low blood pressure. Chlortalidone has a higher risk of side effects than hydrochlorothiazide. While it may be used in pregnancy it is a less preferred option. Specifically it is a thiazide-like diuretic.[11-13] Chlortalidone reduces reabsorption of sodium and chloride primarily through inhibition of the Na+/Cl− symporter in the apical membrane of distal convoluted tubule cells in the kidney. Some of chlortalidone's diuretic effect is also due to inhibition of carbonic anhydrase in the proximal tubule.[14]

The IUPAC Name of Chlorthalidone is 2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide.[15-17]

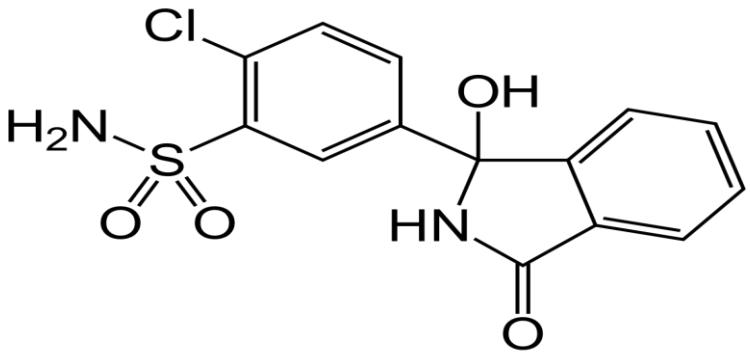


Fig 1: Chemical Structure of Chlorthalidone

II.MATERIALS AND METHODS

Method Development

HPLC Instrumentation &Conditions:

The HPLC system employed was HPLCwith Empower2 Software with Isocratic with UV-Visible Detector.

Standard & sample preparation for UV-spectrophotometer 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 0.1 ml of the above solution into a 10 ml volumetric flask and make up to volume with mobile phase.

Optimized Chromatographic Conditions:

Column: Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. Column.
Mobile Phase :0.1% Orthophosphoric acid: Acetonitrile: Methanol (12:18:70 v/v/v).
Flow Rate : 1.0ml/minute
Wave length : 245 nm
Injection volume :20µl
Run time :07 mins.
Column temperature : Ambient
Sampler cooler : Ambient

Mobile Phase Preparation

Mobile phase was prepared by taking 0.1% Orthophosphoric acid: Acetonitrile: Methanol (12:18:70 v/v/v). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

Sample & Standard Preparation For Theanalysis

25 mg of Chlorthalidonestandard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Method Validation

Accuracy:

Recovery study:To decide the exactness of the proposed strategy, recuperation thinks about were completed by including diverse sums (80%, 100%, and 120%) of unadulterated medication of Chlorthalidone were taken and added to the pre-dissected detailing of fixation 10µg/ml. From that rate recuperation esteems were ascertained. The outcomes were appeared in Table-3.

Precision :

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 deviations were calculated for Chlorthalidone is presented in the Table-4.

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.

Linearity and Range :

Linearity range was found to be 0-70µg/ml for Chlorthalidone.The correlation coefficient was found to be 0.999, the slope was found to be 16660 and intercept was found to be 11860 for Chlorthalidone.

Method Robustness :

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature ($\pm 2^0$C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-39, % RSD < 2%) the developed RP-HPLC method for the analysis of Chlorthalidone (API).

LOD & LOQ :

The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected, but not quantitated. LOD is a limit test that specifies whether an analyte is above or below a certain value. Signal-to-noise ratio of three-to-one is used to determine LOD.

The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ.

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.

7. Estimation of Chlorthalidone in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-8.

Stability Studies

Acid Degradation :

An accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water bath at 600C for 4 hours. Allowed to cool to room temperature. The sample was then neutralized using dilute NaOH solution & final volume of the sample was made up to 100ml with water to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase (after optimizing the mobile phase compositions). This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile. The typical chromatogram shown below is the degradation profile of Chlorthalidone in 0.1N HCl.

Basic Degradation :

An accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of 0.1N NaOH was added to it. & it was refluxed in a water bath at 600C for 4 hours. Allowed to cool to room temperature. The sample was than neutralized using 2N HCl solution & final volume of the sample was made up to 100ml to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase after optimizing the mobile phase compositions. This experiment was repeated several times using same concentration of NaOH such as 0.1N to observe its degradation profile. The chromatogram shown below is the degradation profile of Chlorthalidone in 0.1N NaOH.

Thermal Degradation :

Accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of HPLC water was added to it. Then, it was refluxed in a water bath at 600 c for 6 hours uninterruptedly. After the reflux was over, the drug became soluble and the mixture of drug & water was allowed to cool to room temperature. Final volume was made up to 100 ml with HPLC water to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase.

Photolytic Degradation :

Approximately 10 mg of pure drug was taken in a clean & dry Petridish. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg of the UV exposed drug was transferred to a clean & dry 10 ml volumetric flask. First the UV exposed drug was dissolved in methanol & made up to the mark with mobile phase to get 100 µg/ml solution. Finally this solution was injected into the HPLC system against a blank of mobile phase and chromatogram was obtained.

Oxidation Degradation :

Accurately weighed 10 mg. of pure drug was taken in a clean & dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 100 µg/ml solution. The above sample was injected into the HPLC system.

III.RESULT AND DISCUSSION

Method Development

UV-Spectrophotometer Analysis :

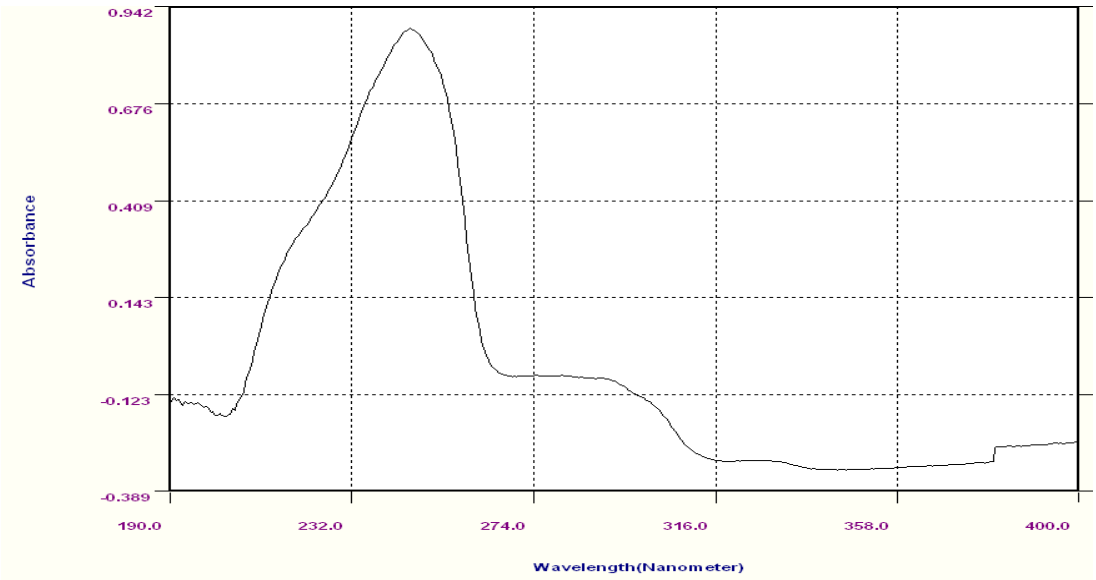


Fig 2: UV spectrum

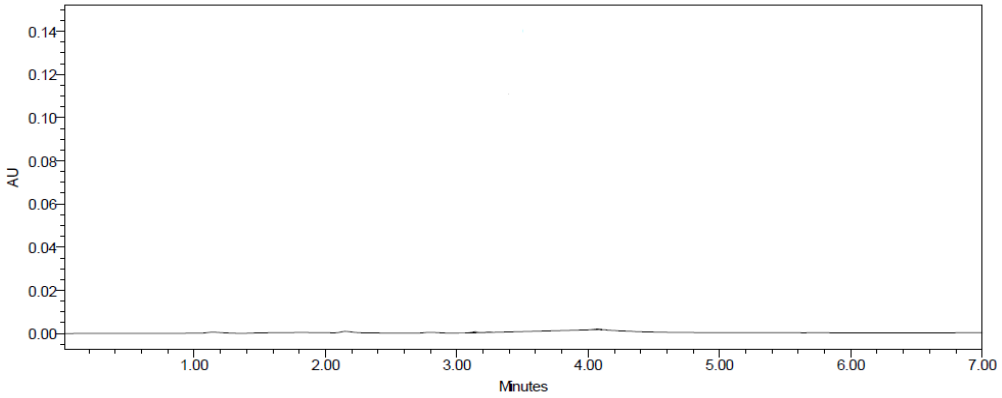
Trials:

Table-1:Trials for Method Development

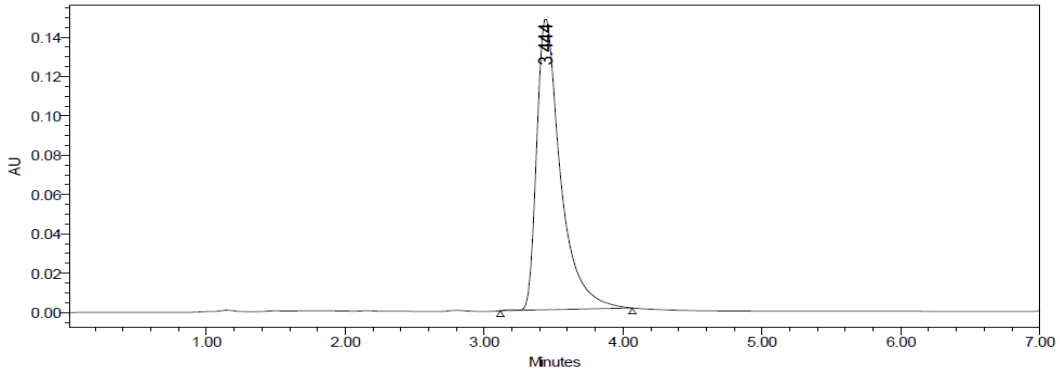
Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. Column	Acetonitrile: Water = 60 : 40	1.0ml/min	245nm	Very Low response	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. Column	Methanol : Water = 70 : 30	1.0ml/min	245nm	Low response	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. Column	Acetonitrile: Methanol = 60 : 40	1.0ml/min	245nm	Tailing peaks	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. Column	Phosphate Buffer : Acetonitrile = 30:70 (pH-3.8)	1.0ml/min	245nm	Resolution was not good	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. Column	0.1% Orthophosphoric acid: Acetonitrile: Methanol (4:14:82 v/v/v)	1.0ml/min	245nm	Tailing peak	Method rejected

Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. Column	0.1% Orthophosphoric acid: Acetonitrile: Methanol (12:18:70 v/v/v)	1.0ml/min	245nm	Nice peak	Method accepted

Optimized Condition :



Chromatogram for Blank Preparation



Optimized Condition

Table 2: Peak results

S.No.	Drug Name	Rt	Peak Area	Tailing Factor	Plate Count
1	Chlorthalidone	3.444	1761430	1.54	5621

Method Validation:

Accuracy :

Table-3: Accuracy Readings

Conc. In ppm	Conc. Found	Peak Area	% Recovery
8	8.042	145847	100.525
8	8.100	146821	101.250
8	8.032	145682	100.400
		Avg.	100.725
		S.D	0.458939
		%RSD	0.455636
Conc. In ppm	**Conc. Found**	**Peak Area**	% Recovery
10	10.030	178975	100.300
10	10.045	179213	100.450
10	10.025	178879	100.250
		Avg.	100.3333

		S.D	0.104083
		%RSD	0.103738
Conc. In ppm	**Conc. Found**	**Peak Area**	% Recovery
12	12.032	212314	100.266
12	12.086	213216	100.716
12	12.052	212658	100.433
		Avg.	100.4717
		S.D	0.227478
		%RSD	0.22641

Precision :
Repeatability

Table-4: Repeatability Results of Precision

HPLC Injection Replicates of Chlorthalidone	Retention Time (Minutes)	Peak Area (AUC)
Replicate – 1	3.475	1765847
Replicate – 2	3.447	1758986
Replicate – 3	3.446	1756245
Replicate – 4	3.447	1761430
Replicate – 5	3.449	1765241
Replicate – 6	3.469	1765864
Average		**1762269**
Standard Deviation		**4057.819**
% RSD		**0.230261**

Intra day &Inter day:

Table-5: Results of Intra day & Inter day

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	7.89	0.95	8.07	0.96
10	10.09	0.76	9.86	0.67
12	11.85	0.95	12.05	0.63

Linearity and Range

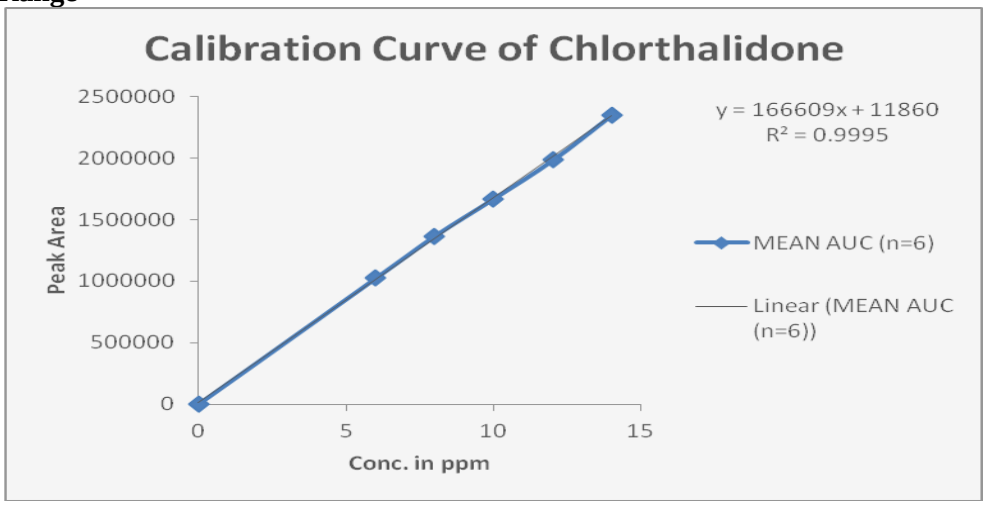


Fig-3:Calibration curve of Chlorthalidone (API)

Table-6: Linearity Results of Chlorthalidone

CONC.(µg/ml)	MEAN AUC (n=6)
0ppm	0
6ppm	1024713
8ppm	1367815
10ppm	1671430
12ppm	1985214
14ppm	2352457

Method Robustness :

Change in parameter	% RSD
Flow (1.1 ml/min)	0.96
Flow (0.9 ml/min)	0.84
Temperature (27^0C)	0.62
Temperature (23^0C)	0.92
Wavelength of Detection (208 nm)	0.76
Wavelength of detection (204 nm)	0.91

LOD and LOQ :

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.08 & 0.24 µg/ml respectively

System Suitability Parameter

Table-7: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.75
2	Asymmetry	T ≤ 2	Chlorthalidone =0.17
3	Theoretical plate	N > 2000	Chlorthalidone =5897
4	Tailing Factor	T<2	Chlorthalidone =1.59

Estimation of Chlorthalidone in Tablet Dosage Form

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)
Hythalton (100 mg) (Nicholas Piramal India Ltd.)	100mg	99.875 (± 0.654)	99.86 (± 0.451)

Stability Studies

Acid Degrdation :

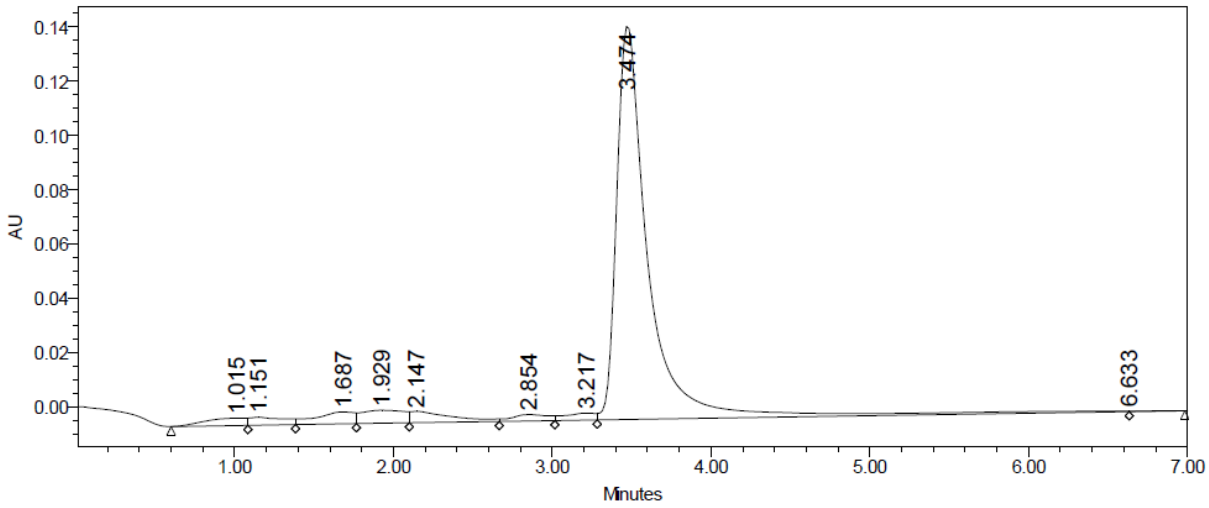


Fig.4: Acidic degradation

Alkaline Hydrolysis:

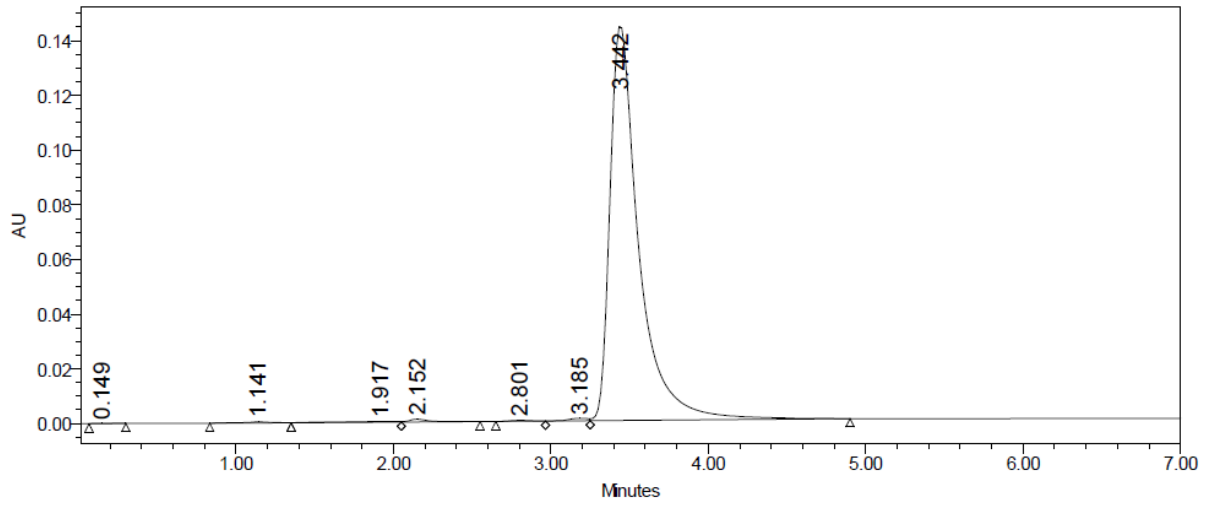


Fig.5: Basic degradation

Thermal Degradation:

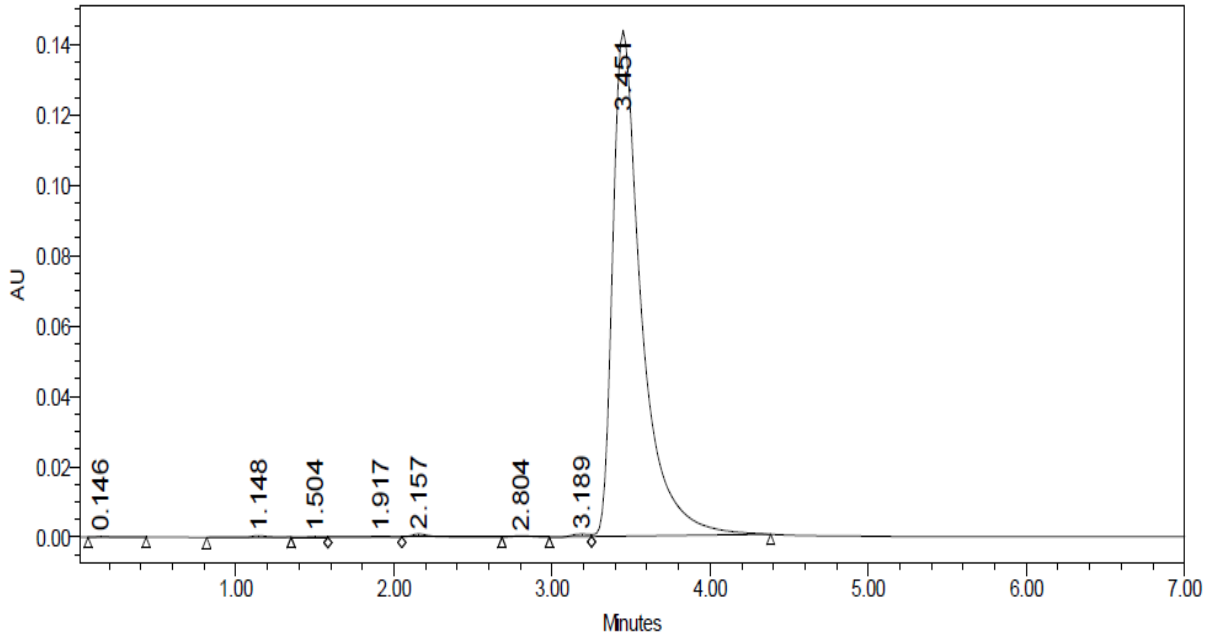


Fig.6: Thermal degradation studies

Photolytic Degradation:

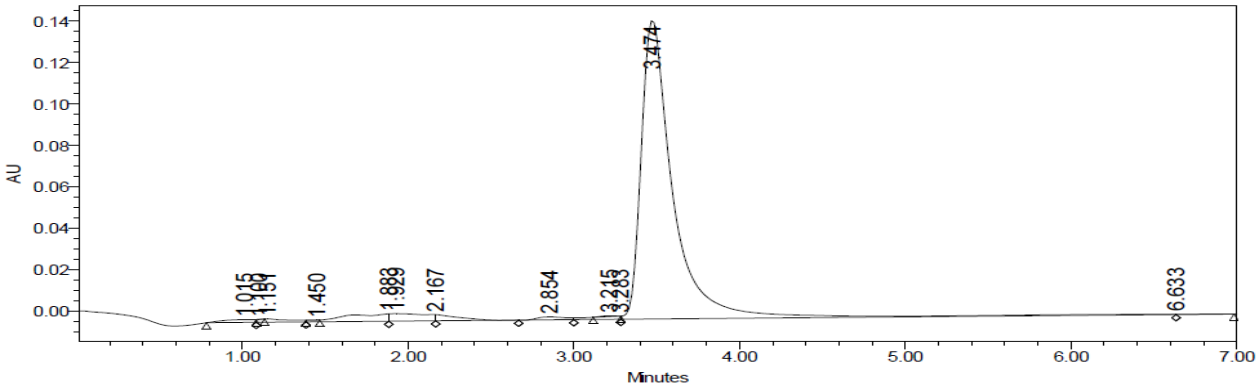


Fig.7:- Photolytic degradation.

Oxidation With (3%) H_2O_2:

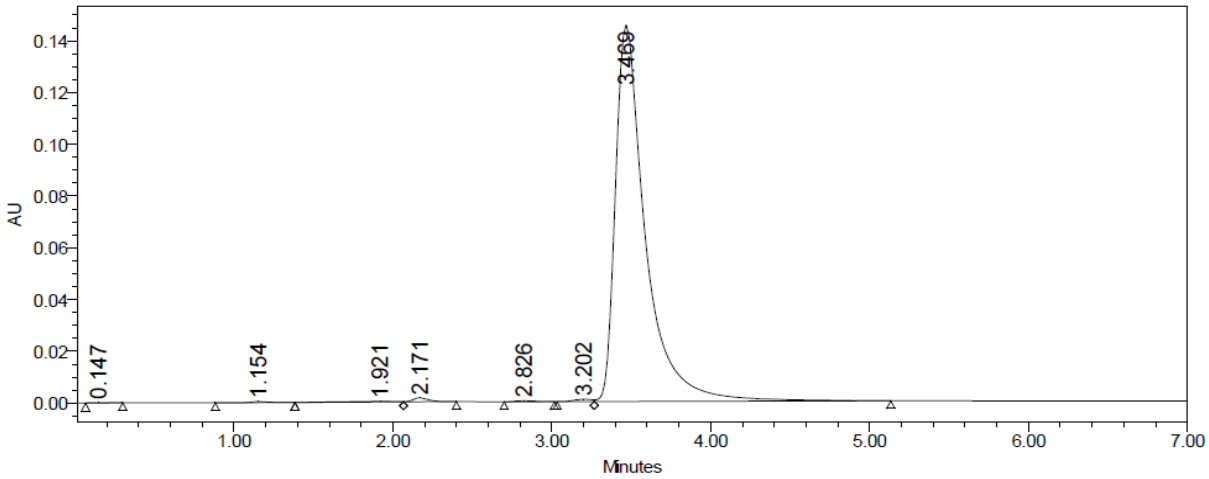


Fig.8: Peroxide degradation

Table-9: Results of forced degradation studies of Chlorthalidone API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	88.92	11.08	100.0
Basic Hydrolysis (0.I M NaOH)	24Hrs.	96.51	3.49	100.0
Thermal Degradation (50 ^{0}C)	24Hrs.	96.33	3.67	100.0
UV (254nm)	24Hrs.	89.18	10.82	100.0
3 % Hydrogen Peroxide	24Hrs.	97.44	2.56	100.0

IV.CONCLUSION

A sensitive& selective RP-HPLC method has been developed & validated for the analysis of Dapagliflozin API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Dapagliflozin in different formulations.Finally it was concluded that the method is simple,sensitive and has the ability to separate the drug fromdegradation products and excipients found in thedosage form.

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