“Analytical Techniques for the Estimation of Pharmaceutical Drugs in Pure and Tablet Dosage Forms”

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Abstract :

In this study , the author has developed a HPTLC method for the estimation of Chlorthalidone in Commercial brand CTD-12.5 of Chlorthalidone. The values obtained suggested that the proposed HPTLC method was simple , precise , rapid and robust for determination of Chlorthalidone . The mobile phase was simple to prepare and economical . The authors then validated the method as per ICH guidelines and correlated the obtained values with standard values. Satisfactory result were obtained .

KEYWORDS : Chlorthalidone , Validation of HPTLC , Method Development .

INTRODUCTION :

Chlorthalidone having C14 , H17 , CIN2 , O4S . The Molar Mass of the Chlorthalidone is 338.766g/mol . Chlorthalidone drug is Prescribed in the Treatment in High blood pressure (Hypertension) , swelling including that due to heart , liver failure , and nephritic syndrome and diabetes insipidus and renal tubular acidosis . Common side effects include low blood potassium . Low blood sodium , high blood sugar .

Chlorthalidone was patented in 1975 and come into medical use in 1690 . It is available as generic medication . in 2017 it was the 173th most commonly

prescribed medication in the united states, with more than three million prescription .

It is used in Medical use in High blood Pressure , Left ventricular hypertrophy , swelling , Bone fracture prevention , menieres disease , Diabetese insipidus .

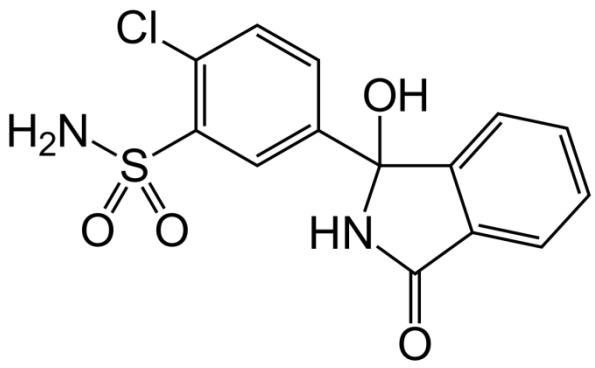

Fig no 1 : Structure of Chlorthalidone

Material And Method :

1.1 Preparation of Standard stock Solution :

Standard stock solution of drug prepared by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000mg/ml. From the standard stock solution , working standard solution was prepared containing 500 mg/ml of Chlorthalidone .

1.2 Selection of detection wavelength :

From the Standard stock solution (1000 mg/ml) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra was obtained . It was observed that the drug showed considerable absorbance at 275 nm.

Respective UV spectrum of Chlorthalidone is Shown in fig no 2.

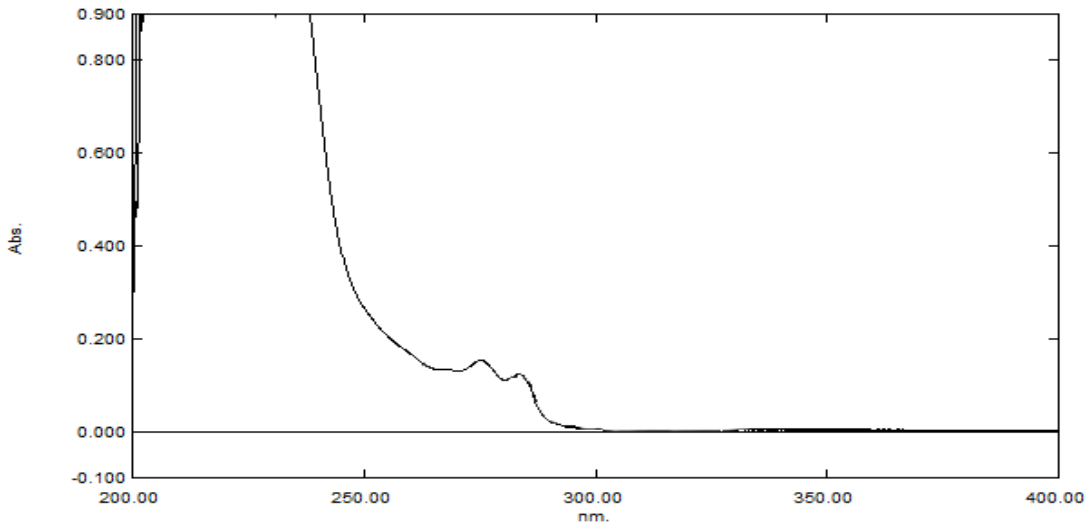

Fig 1 : The UV spectrum of Chlorthalidone (10mg/ml)

1.3. Selection of Mobile phase and Chromatographic conditions :

Chromatographic separation studies were carrid out on working standard of Chlorthalidone 1000 ng/band . Initally , trials were carried out using solvants in various proportions on normal TLC plate to obtain the desired Rf

And shape for drug peak. After few trials , Ethyl acetate : Methanol (7.5:2.5v/v) was chosen as the mobile phase , Which gave acceptable peak parameter . Other chromatographic condition like chamber saturation time,run length ,Sample application volume were optimized.

1.4. Preparation of Sample solution :

For determination of the content of Chlorthalidone in Chlorthalidone tablets (Lable clain:12.5mg Chlorthalidoneper tablet),twenty tablets werw weighed; average weight was determined and werw finely powdered.A quantity of powder equivalent to 10mg of Chlorthalidone was transferred to a 10 ml volumetric flask containing 5 ml of methanol. The mixture was ultra sonicated for 10 min and the resulting sample stock solution was filtered with Whatman filter paper no 41 and the volume was made up with the methanol. 5.0 ml of this solution was diluted to 10 ml with the methanol to prepare final stock solution of 500mg/ml.

1.5 . **Densitogramand system suitability parameter of drug :**

Solution of Chlorthalidone (500 mg/ml) was prepared. 2 ml (1000 mg/band) of solution was applied on pre-activated TLC plate with the help of Hamilton syringe (100ml) , using Linomat 5 sample applicator . The development chamber was saturatedwith mobile phase for 15 min. The spotted plate was placed in saturated chamber and developed up to 80 mm distance. The plate was dried and was scanned over 90mm distance at 275 nm. The retention factor was found to be 0.43 ± 0.07. Representative densitogram of Chlorthalidone (1000 ng/band) is shown in fig.2

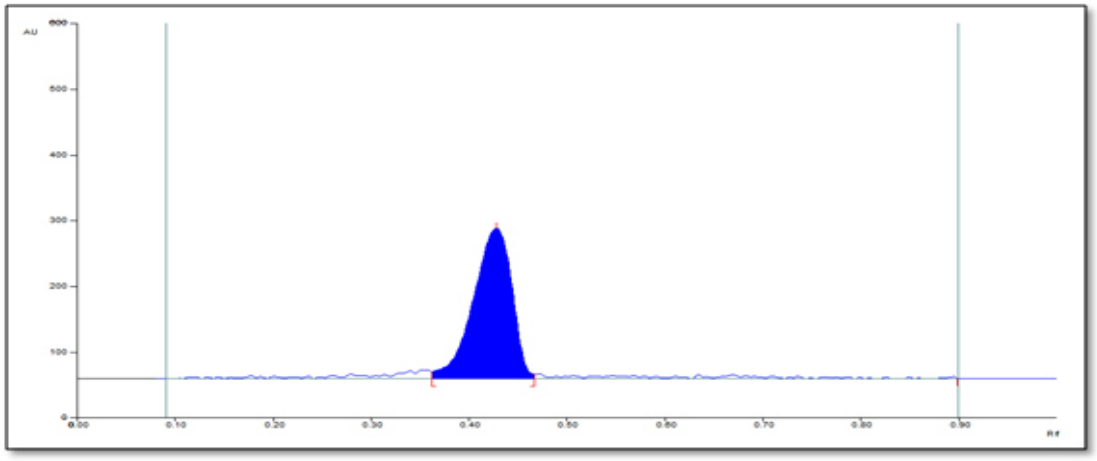

Fig 2 : Densitogram of standard solution of Chlorthalidone (1000 ng/band)

Table 2 : System suitability parameters for chlorthalidone :

Drug	Conc. (ng/band)	Rf	Area	Asymmetry
Chlorthalidone	1000	0.43± 0.07	8447.7	0.86

1.6. Summary of chromatographic parameters selected :

Chromatograpic parameter are summarized in Table 3

Table No 3 Chromatographic Parameters :

Sr. No	Parameter	Conditions used for Analysis
1	Stationary phase	TLC aluminium plate precoated with silica gel 60 F254
2	Mobile phase	Ethyl acetate:MeOH(7.5:2.5v/v)
3	Detection Wavelength	275 nm
4	Saturation time	15 min
5	Band width	6 mm

2.VALIDATION OF ANALYTICAL METHOD :

2.1. Specificity

The specificity of the method was ascertained by peak purity profile studies.The peak purity values were found to be more than 0.998, indicating the no interference of any other peak of degradation product , impurity or matrix.

2.2 . Linearity :

From the standard stock solution (1000 mg/ml) of Chlorthalidone, Solution was prepared containing 500 mg/ml of Chlorthalidone . This Solution was further used for spotting . Six replicates per concentration were spotted . The linearity (relationship between peak area and concentration) was determined by analyting six concentrations over the concentration range 500-3000 mg/ band to obtain calibration curve. The results found to br linear with regression equation y=5.5173x + 2453.8 and R2 = 0.9928.

The result obtained are shown in Table 4. The calibration curve is shown in fig 3 .

Table No 4 : Linearity study of Chlorthalidone :

Replicate	Concentrations of Chlorthalidone (ng/band)					
	500	1000	1500	2000	2500	3000
	Peak area					
1	4688.2	8447.7	10625.5	13595.5	16565.1	18059.5
2	4611.6	8570.7	10503.3	13980.1	16551.3	18719.9
3	4677.7	8596.6	10856.6	13513.9	16301.8	18865
4	4681.5	8543.5	10575	13977.3	16231.4	18581.1
5	4610.5	8572.8	10578.4	13469.7	16670.5	18564.5
6	4694.4	8531.1	10547.3	13528.8	16552.6	18594.5
Avg	4660.650	8543.733	10614.350	13677.550	16478.783	18564.077
SD	38.844	52.439	125.282	236.740	171.718	272.268
% RSD	0.833	0.614	1.180	1.731	1.042	1.467

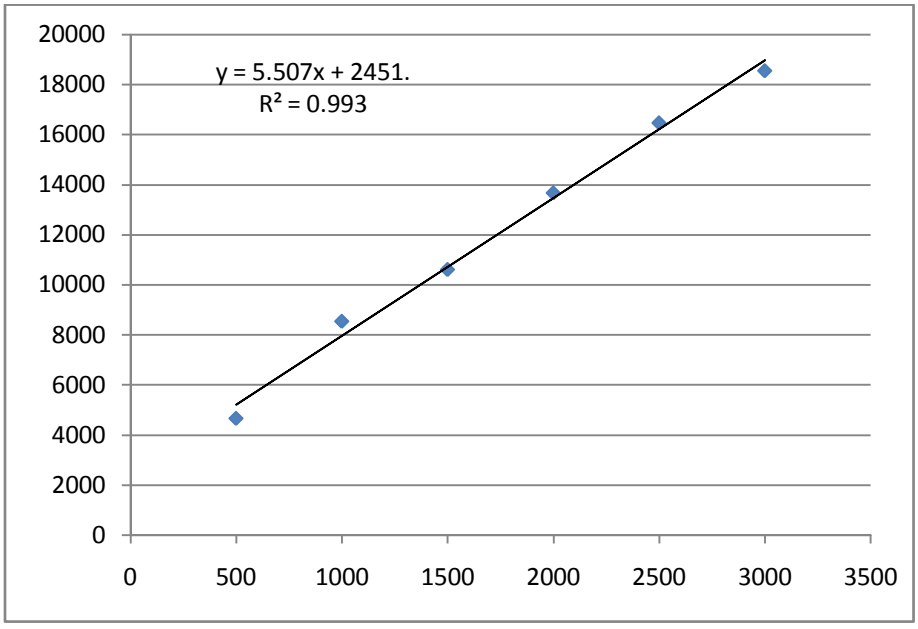

Fig 3 : Calibration curve of Chlorthalidone (500-3000 ng/band) reference standard

2.3 Range :

Chlorthalidone = 500-3000ng/band

2.4 : Precision :

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies 3 replicates of 3 concentrations were analysed on the same day , and % RSD was calculated . For the interday variation studies, 3 concentrations were analysed on 3 consecutive days and % RSD was calculated . For interaday precision and interday precision results obtained are shown in Table 5 and 6.

Table 5 : Intraday variation studies data for Chlorthalidone :

Conc. (ng/band)	Area	% recovery	Average	SD	%RSD
1000	8072.8	102.079	101.422	0.614	0.605
	8031.1	101.322			
	8005.9	100.864			
1500	10578.4	98.385	98.734	0.513	0.520
	10587.3	98.493			
	10655.9	99.323			
2000	13528.8	100.576	101.343	0.669	0.660
	13646.8	101.648			
	13664.2	101.806			

Table 6 : Interday variation studies data for Chlorthalidone :

Conc. (ng/band)	Area	% recovery	Average	SD	%RSD
1000	1010.785	101.078	101.114	0.170	0.168
	1012.982	101.298			
	1009.641	100.964			
1500	1484.327	98.955	99.375	1.254	1.261
	1511.765	100.784			
	1475.775	98.385			
2000	2023.641	101.182	100.733	0.395	0.392
	2008.823	100.441			
	2011.529	100.576			

2.5 Limit of Detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ are calculated from the formula :

$$LOD = \frac{3.3\ \sigma}{S} \qquad LOQ = \frac{10\,\sigma}{S}$$

Where,

σ = Standard deviation of Y intercept;

S = Average of slope of the calibration curve

Table 7 : LOD and LOQ of Chlorthalidone

Method	Avg slope	S.D	LOQ (ng/band)	LOD(ng/band)
Using S.D of y-intercept	5.523	94.028	170.24	56.18

2.6 Assay:

Chlorthalidone 12.5 mgtablet (Niksan Healthcare) formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. 2 µl volume of sample solution was applied and area was recorded. Basic concentration of sample chosen was 1000 ng/band from tablet solution. Concentration and % recovery was determined from linear equation. Assay results obtained are shown in Table 8.

Table 8: Assay of marketed formulation

Sr. No.	Peak area	Amount recovered (ng/band)	% recovery
1	7999.8	1007.534	100.753
2	7947.6	998.055	99.806
3	8003.2	1008.152	100.815
4	7993.8	1006.445	100.644
5	7983.4	1004.556	100.456
6	8000.7	1007.698	100.770
Mean	7988.083	1005.407	100.541
SD	21.064	3.825	0.382
%RSD	0.264	0.380	0.380

2.7 Accuracy:

To check accuracy of the method, recovery studies were carried out by spiking the standard drug to the tablet solution, at three different levels 50, 100 and 150%. Basic concentration of sample chosen was 1000 ng/band. % recovery was determined from linear equation. Accuracy results obtained are shown in Table 9

Table 9: Accuracy studies of Chlorthalidone

Level	Amount of sample taken (ng/band)	Amount of standard spiked (ng/band)	Area	Amount recovered (ng/band)	% recovery (Mean ±%RSD)
50%	1000	500	10784.6	1513.218	100.390 ±0.446
			10735.2	1504.248	
			10712.2	1500.071	
100%	1000	1000	13648.2	2033.211	100.992 ±1.227
			13658.5	2035.081	
			13417.1	1991.246	
150%	1000	1500	16199.5	2496.494	100.564 ±0.882
			16433.6	2539.003	
			16256.4	2506.826	

2.8 Robustness :

Robustness of the method was determined by carrying out the analysis under conditions during which chamber saturation time was altered. Time was also changed from spotting to development and development to scanning and the effects on the area were noted. It was found that method is robust. The results obtained are shown in Table10

Table 10: Robustness study

Sr. No.	Parameters	Variation	%RSD
1.	Time from application to development	0 min	1.465
		30 min	1.111
		60 min	0.759
2.	Time from development to scanning	0 min	1.477
		30 min	1.464
		60 min	0.879
3.	Saturation Time	15 min	0.614
		13 min	0.942
		17 min	0.858

3.Summary of validation study

Table 11: Summary of Validation Parameters

Sr. No.	Validation parameters	Chlorthalidone
1.	Linearity equation R^2 Range	y = 5.507 x + 2451.309 R^2= 0.993 500-3000ng/band
2.	Precision	(%RSD)
	Intraday	1.417
	Interday	1.028
3.	Assay	100.541±0.380
4.	Accuracy	
	50	100.390 ±0.446
	100	100.992 ±1.227
	150	100.564 ±0.882
5.	Limit of detection	56.18ng/band
6.	Limit of quantitation	170.24ng/band
7.	Specificity	Specific
8.	Robustness	Robust

Conclusion :

It includes that the developed method is simple , accurate and precise and suitable for the routine analsis , The developed method werw validated as per ICH guidelines and were found to be within limit.

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