ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CHLORTHALIDONE IN BULK AND DOSAGE FORMS BY UV SPECTROPHOTOMETRY

Ankush Gupta[1], Prof. (Dr.) Alka N. Choudhary[2]

[1]School of Pharmaceutical Sciences, SGRR University, Dehradun, Uttarakhand, India

[2]Dean, School of Pharmaceutical Sciences, SGRR University, Dehradun, Uttarakhand, India

ABSTRACT

A new, uncomplicated, errorfree, expeditious and precise spectrophotometric technique has been evolved for validation of Chlorthalidone in bulk and dosage forms. This technique was established on measurement of absorptivity at 275 nm at the scale of 5-25 µg/mL using Ethanol as solvent. The results have been analyzed and validated according to ICH guidelines. The spectrophotometric response was achieved and was recognized to be linear at the concentration scale of 5-25µg/mL with a correlation coefficient of 0.9992. The correctness was found to be 99.73%. The precision was found to be 0.46. The LOD and LOQ was 0.2407 and 7.90respectively. The analyzedtechniqueshows beyond doubt that the method is repeatable and particular for the ascertainment of the above drug. These methods can be embraced for the regime assay examination of chlorthalidone in API as well as dosage forms.

Keywords: *Spectrophotometric, Chlorthalidone, Ethanol and validation.*

INTRODUCTION

Chlorthalidone (CTD) (first inaugurated in Switzerland in 1959) is a sulphanylbenzophenone derivative[2-chloro-5-(1-hydroxyl-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide]. It act as a diuretic agent employed in the therapy of oedema correlated with clogged heart failure contrast with other medications similar to thiazide class. Chlorthalidone has an extendedperiod of action but an indistinguishable diuretic outcome at topmost therapeutic doses.[1,2]

ANALYTICAL METHOD DEVELOPMENT

Analytical method development and validation has a vital role in the development and manufacturing of pharmaceutical dosage forms. Method development is a procedure for proving that an analytical method is admissible for use to estimate the concentration of an Active Pharmaceutical Ingredient (API) in a pharmaceutical dosage form. As number of drugs were introduced in the market due to which analytical methods and for such drugs are not available. So, it is mandatory to develop newer methods for such drugs.[3,4,5]

MATERIALS AND METHODS

Materials: Chlorthalidone working standard drug was obtained from Synokem Pharmaceuticals, Haridwar, Uttarakhand. Analytical grade solvents were supplied by Changshu Hongsheng Fine Chemical Co. Ltd. Freshly prepared samples were used for analytical method development.

Equipment: The UV- Spectrophotometry (Model no. GS2281) with data processing system (Carry 60 UV-VIS) by Agilent Tech. was used. The observations of samples were analysed in 1cm Quartz cell against blank in the range of 200-400 nm. The Digital weighing balance of Shimadzu Instrument Pvt. Ltd was used for weighing the sample. An ultrasonicator bath (SONAR) was used for sonicating the drug sample.

METHOD VALIDATION

The propound technique was validated in accordance with ICH standards.

Preparation of calibration curve

Fresh aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 ml were pipetted out of the standard stock solution within a 10 ml volumetric flask and the final volume was make up to 10 ml with ethanol to get the resulted concentration in the range of 5-25 µg/ml. The solutions were examined using a UV-spectrophotometry device for the wavelength range of 200-400 nm, and a strong peak was detected at 275 nm. The linearity was perceived over the scale of 5-25 µg/ml. Absorbance was measured at 275 nm with ethanol as a blank. Figure 1 shows wavelength maxima and figure 2shows a calibration curve that combines concentration and absorbance.

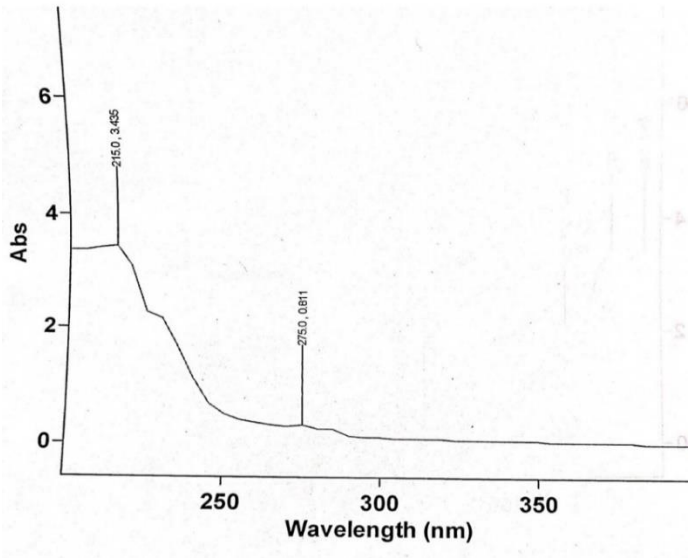

Fig. 1 Wavelength maxima of chlorthalidone

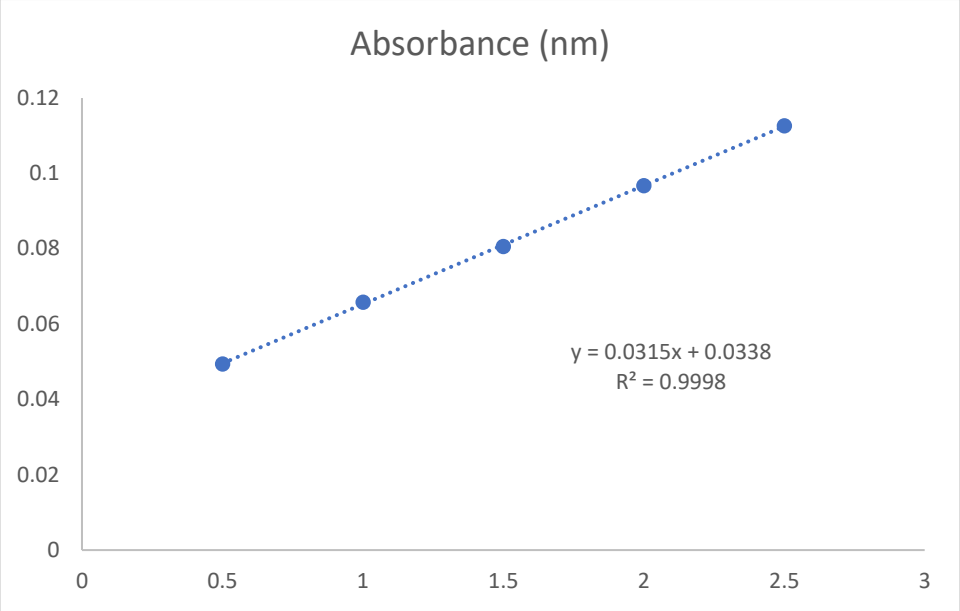

Fig. 2: Calibration curve of Chlorthalidone

Linearity

The developed approach was validated in accordance with ICH recommendations. In which 0.5, 1, 1.5, 2, 2.5ml of standard Chlorthalidone solutions were prepared in a series of 10ml volumetric flask. The volume was made up to mark to achieve concentration of 5, 10, 15, 20, 25 µg/ml. Then the transmittance was measured and graph was plotted between concentration and absorbance. Table 1depicts a plot of absorbance vs concentration. The graph plot follows the Beer's range of 5-25 g/mL with a correlation coefficient (R2) of 0.9992.

S. No.	Concentration µg/mL	Absorbance	E1cm1%	Absorptivity	Molar Absorptivity
1	5	0.2931	586.2	58.62	19813.5
2	10	0.4798	479.8	47.98	16217.2
3	15	0.6420	428.0	42.80	14466.4
4	20	0.8069	403.4	40.34	13634.9
5	25	0.9671	386.8	38.68	13073.8
		Mean	456.84	45.68	15441.16

Table 1: Linearity studies of Chlorthalidone.

Repeatability

Pipette out 1, 1.5, 2ml operating solution and transmit into a sequence of nine, 10ml volumetric flasks. Dilute it to 10ml with ethanol to acquire 10, 15, 20 µg/ml solutions respectively. Transmittance of the aftermath solutions was computed at 275nm employing ethanol as blank. The result obtained as shown in table 2.

Concentration (µg/mL)	Absorbance	Observed concentration (µg/mL)	Mean concentration (µg/mL)	Standard deviation	% Relative standard deviation
10	0.4773	10.1	10.1	0.070	0.69
	0.4701	10.0			
	0.4722	10.1			
15	0.6408	15.1	15.1	0.070	0.46
	0.6375	15.0			
	0.6419	15.1			
20	0.8083	20.1	20.1	0.003	0.014
	0.8050	20.0			
	0.8079	20.1			
			Mean	0.047	0.388

Fig. 2: Repeatability studies.

Precision

It is defined as the proximity of consensus betwixt a sequence of measurements obtained during sampling. This parameter includes intraday and inter-day precisions.

Intraday

Pipette out 1,1.5 and 2ml operating solution and transmit into distinct 10 ml volumetric flasks and compose the volume to 10ml with ethanol to acquire the concentrations of 10, 15 and 20µg/ml individually. Transmittance of the aftermath solutions was computed at 275nm employing ethanol as blank. Such three alteration were executed within a day after every 3hrs interval. The result was shown in table 3.

Conc. (µg/mL)	**Absorbance**			**Observed conc. (µg/mL)**			**Mean conc. (µg/mL)**	**Standard deviation**	**Relative standard deviation**
	0hrs	3hrs	6hrs	0hrs	3hrs	6hrs			
10	0.4733	0.4767	0.4759	10.1	10.2	10.2	10.2	0.0707	0.69
15	0.4767	0.6475	0.6463	15.1	15.3	15.3	15.2	0.038	0.25
20	0.4759	0.8083	0.8050	20.2	20.1	20.0	20.1	0.031	0.154
							Mean	0.046	0.36

Table 3: Intraday precision

Interday

Pipette out 1, 1.5, and 2ml of operating solution into separate 10ml volumetric flasks. Dilute them all to 10ml with ethanol to get solutions with prepared concentrations of 10, 15, and 20g/ml. At 275nm, the absorbance of the following solutions was calculated employing ethanol as a blank. Such tripartite examinations were executed during the day one, day two (24 hours), and day three (48 hrs) interludes. Table 4depicts the end product.

Concentration (µg/mL)	Absorbance			Observed concentration (µg/mL)			Mean conc. (µg/mL)	Standard deviation	Relative standard deviation
	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs			
10	0.4701	0.4666	0.4633	10	9.9	9.8	9.9	0.10	1.01
15	0.6375	0.6369	0.6341	15	15	14.9	15.0	0.070	0.46
20	0.8117	0.8061	0.8083	20.2	19.9	20.1	20.1	0.158	0.078
							Mean	0.109	0.516

Table 4: Interday precision

Analysis of marketed formulation

20 tablets were precisely weighed and reduced to fine powder with the help of laboratory mortar and pestle. A precisely weighed powder sample equal to 10mg of Chlorthalidone was transmitted to 100ml volumetric flask and 30 ml ethanol was append to it. Sonicate it for 10 minutes and solution was strained using whattman filter paper no. 41. Then the filtrate was further mixed with ethanol to acquire last concentration of 1000 µg/mL. From this solution 10µg/mL was prepared and absorbance of sample was taken at 275nm and the results are shown in table 5.[6.7]

Drug sample	Label amount (mg)	Amount found (µg/ml)	% Drug content	Standard deviation	Relative standard deviation
1	12.5	12.48	99.84	0.56	0.563
2	12.5	12.39	99.12		
3	12.5	12.37	98.96		
4	12.5	12.47	99.76		
5	12.5	12.24	97.92		
	Mean	**12.39**	**99.32**		

Table 5: % Assay of Chlorthalidone

Accuracy

Pipette out 1.5ml of pennant solution and transmit to 10ml volumetric flasks. Such moves were planned nine times. To make 27g/ml solutions, spike three volumetric flasks with 1.2ml of working solution (see Formulation) and dilute one and all to 10 ml with ethanol. To make 30g/ml solutions, spike three more solutions with 1.5ml of working solution (from Formulation) and dilute one and all to 10 ml with ethanol. Mix last three of the solutions with 1.8ml of working solution (Produced from Formulation) and then dilute one and all to 10 ml with ethanol to get 33µg/ml solutions. Absorbance of the aftermathprepared dilutions was computed at 275nm using ethanol as blank. The obtained results were shown in table 6.

Recovery at	Nominal conc. µg/mL	Absorbance	Observed conc.	% Recovery
80	15+12=27	1.0390	27	100
80	15+12=27	1.0360	26.9	99.6
80	15+12=27	1.0320	26.9	99.6
100	15+15=30	1.1366	29.9	99.6
100	15+15=30	1.1401	30	100
100	15+15=30	1.1358	29.9	99.6
120	15+18=33	1.2371	32.9	99.6
120	15+18=33	1.2405	33	100
120	15+18=33	1.2359	32.9	99.6
			Mean	99.73

Table 6: Repeatability studies or Accuracy.

Limit of Detection and Limit of Quantification

Limit of Detection and Limit of Quantification were computed as stated by ICH guidelines. LOD was contemplate as the little concentration that can be observed with a sufficient degree of statistical significance, although LOQ was the little concentration of a substance that is feasible to be determined by means of a stated analytical procedure. The results are shown in table 7.[8,9]

S. No.	Concentration	Absorbance	LOD	LOQ
1	5	0.2931	0.2407	7.90
2	10	0.4798		
3	15	0.6420		
4	20	0.8069		
5	25	0.9671		

Fig. 7: LOD and LOQ.

RESULT AND DISCUSSION

The obtained method has been validated for accuracy, precision, repeatability and specificity. The nominal concentration of pennant solutions was approximately 5-25µg/ml. The suggested method was established to be linear with a linear correlation coefficient of 0.9998 and the linear regression equation was y=0.0315x + 0.0338 (fig. 2). The linearity of the concentration degree at which Chlorthalidone can be reliable was5-25µg/ml. The mean recoveries were 99.73% substantiated the technique as accurate (Table 6). The repeatability, intraday, inter-day precision, specificity and accuracy, RSD was less than 2%. Potency assay of dosage forms of Chlorthalidone were executed by the proposed technique. The brand products reach the pennant criteria with the new analytical technique. Specificity is the potential of the describedtechniquefurnish data on specificity for their assessment in the presence formulation excipients. The absorbance acquired with the combination of the excipients exhibit no interference with the absorbance ofpennant. The percent assay of Chlorthalidone was found to be 99.39 in marketed product (CTD 12.5mg) in table 5.

CONCLUSION

The developed UV-Spectrophotometric method was established to be uncomplicated, accurate, pinpoint, reproducible and sensitive. The suggested technique was initiate appropriate for determination of Chlorthalidone in bulk and dosage forms in the absence of any intercession from the excipients. This technique can be efficaciously employed for the regime analysis of Chlorthalidone in bulk.

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