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Stress degradation studies of hydrochlorothiazide and development of validated method by UV spectroscopy

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Abstract: To develop a simple, precise, accurate, and stability indicating a UV-method for estimation of Hydrochlorothiazide (HCT) in bulk and formulated dosage form. The drug was also subjected to stress degradation at different conditions recommended by the International Conference on Harmonization (ICH). The samples are generated and used for the degradation studies. The λ_{max} of the HCT was found to be 273 nm.The linearity of calibration curve (Absorbance Vs Concentration) in pure solution was checked over the concentration ranges of about 5-30 µg/mL with the correlation coefficient higher than 0.99. The regression equation of the curve was Y = 0.598x + 0.0042. % RSD was found to be within the limit as per ICH guidelines. The obtained percentage recovery of HCT was found to be within the limit 100% ± SD. Stress degradation studies revealed that it was within the limit (5-20%).

Keywords: UV Spectroscopy; Hydrochlorothiazide; Stress degradation; validation

1. Introduction

Hydrochlorothiazide (HCT) is chemically 6-chloro-3, 4-dihydro-2h-1, 2, 4-bezothiadiazine-7-sulphonamide-1, 1-dioxide (Figure 1). It is used in the treatment of hypertension. HCT inhibits active chloride reabsorption at the distal tubule via the sodium chloride co-transporter, resulting in increased excretion of sodium chloride and water. The antihypertensive mechanism of HCT is less well understood although it may mediated through its action on carbonic anhydrases in the smooth muscle (or) through its action on the large conductance calcium activated potassium(KCa) channel, also found in the smooth muscle. This results in an increase in potassium excretion via the sodium potassium exchange mechanism.[1-3]

Extensive literature survey was carried out which revealed that there is no work carried out especially on Hydrochlorothiazide in forced degradation studies using UV Spectrophotometry.[4-8] The specific aim of the research was to develop a UV method for the forced degradation studies of HCT in bulk and formulated dosage form and to validate the proposed methods in accordance with ICH guidelines for the intended analytical application.[9-10]

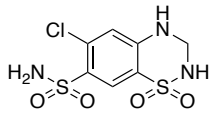

Figure 1. Structure of Hydrochlorothiazide

2. Result and Discussion

In the present work, we have developed a newer, simple, accurate and cost effective UV-Spectrophotometric method for the effective determination of HCT in bulk and formulated Tablet dosage form. The $\boldsymbol{\lambda}_{max}$ of HCT was found to be 273 nm. The percentage purity of HCT was found to be 99.84% w/w. The calibration plot for HCT was observed to be linear in the range of 5-30 µg/mL and the correlation coefficient was found to be 0.999. In precision study it was found that %RSD was less than 2% which indicated that the proposed method has good reproducibility. In accuracy study the % recovery of HCT in bulk drug samples were ranged 100.44%, 101.04% & 101.50% which indicate that the method was accurate. Ruggedness study it was found that %RSD was less than 2%. This indicates that the proposed method was accurate. LOD and LOQ, the Limit of detection (LOD) of HCT was found to be13.56 µg/mL respectively, LOQ of HCT was found to be 34.88 µg/mL.

3. Experimental

Materials and methods: UV-Visible spectrophotometer (LAB India) equipped withUV detector 1.0 cm matching quartz cells was used. Hydrochlorothiazide (HCT) was obtained from Saimirrainno Pharm Pvt Ltd, Chennai, Tamilnadu, India. Sodium hydroxide (AR Grade) was obtained from nice chemical (India) Pvt Ltd.

3.1. Method development

Selection of wavelength: The quantity containing 100 mg of HCT were taken in 100 mL standard flask, and the volume was made up to the mark with 0.1*N* NaOH to obtain 1000 µg/mL. From which 10 mL of solution was taken and diluted to 100 mL and made up to volume to

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obtain 100 µg/mL. From the 100 µgmL solution, 10 mL was taken and transferred into 100 mL standard flask, and the volume was made up to the mark with 0.1*N* NaOH to obtain 10 µg/mL concentration of HCT. The above solution was scanned over range of 200-400 nm (Figure 2.)

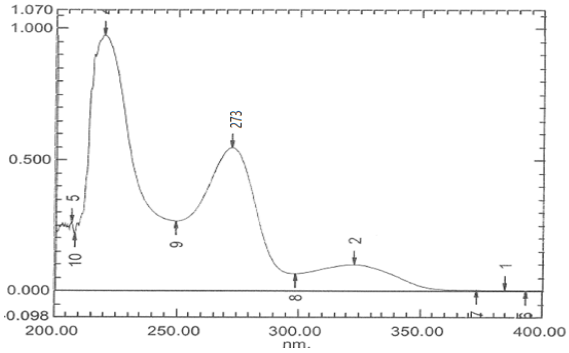

Figure 2. UV-Spectra of Hydrochlorothiazide

Standard preparation: The quantity containing 100 mg HCT was taken into 100 mL clean dry standard flask 0.1*N* NaOH was added and the volume was made up to the mark to obtain 1000 µg/mL and used as stock solution. From the stock solution 1 mL was pipetted out into a 100 mL standard flask and make up to the mark with 0.1*N* NaOH to obtain 10 µg/mL concentration.

Sample preparation: 20 tablets were weighed and powder it, a powder equivalent to 100 mg of HCT was taken into a 100 mL clean dry standard flask 50 mL 0.1*N* NaOH was added and sonicated, the volume was made up to the mark to obtain 1000 µg/mL, further it was diluted with 0.1*N* sodium hydroxide to obtain 10 µg/mL.

3.2. Method Validation

Linearity: The linearity of the methods is determined at six concentration levels ranging from 5-30 µg/mL. The correlation co-efficient of HCT was found to be 0.999 respectively (Figure 3, Table 1).

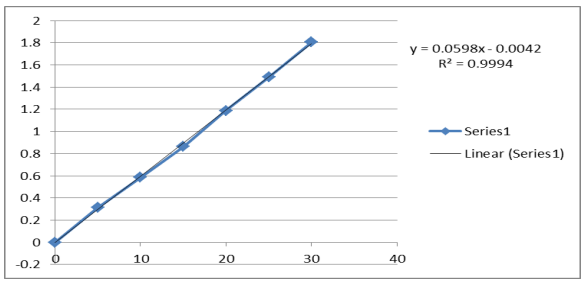

Figure 3. Calibration curve of Hydrochlorothiazide

Table 1. Linearity for Hydrochlorothiazide (HCT)

Concentration (µg/mL)	Absorbance of HCT	Statistical analysis of HCT
5	0.3142	Slope = 0.0598 Correlation co efficient =0.999
10	0.5863	
15	0.8642	
20	1.1864	
25	1.4898	
30	1.8052	

Accuracy: The accuracy of the method was determined by standard addition method at three different concentrations (80%, 100% and 120% concentration) by adding a known amount of standard.

The percentage recovery of HCT was found to be 101.50, 100.44, and 100.04% from 80, 100 and 120% sample solutions, respectively. The obtained percentage recovery of HCT was found to be within the limit. This indicates the proposed method was more accurate (Table 2).

Table 2. Accuracy data for Hydrochlorothiazide (HCT)

Amount (µg/mL)	Level		
	80%	100%	120%
Present	8.050	10.280	12.560
Added	10.15	10.05	10.02
Found	18.22	20.33	22.58
Recovered	10.75	10.22	10.88
%Recovery	101.50	100.44	101.04
%RSD		0.013288	

Precision: The Precision method was determined by performing at intraday and interday precision. % RSD was found to be within the limit as per ICH guidelines (Table 3 & 4).

Table 3. Intraday analysis of Hydrochlorothiazide

Stat	HCT (µg/mL)	
	15	20
	0.8999	1.1864
	0.8982	1.1698
	0.8897	1.1687
Avg.	0.895933	1.174967
S.D	0.005465	0.009917
%RSD	0.0069949	0.00844

Table 4. Interday analysis of Hydrochlorothiazide

Stat	HCT (µg/mL)			
	Analysist 1		Analysist 2	
	15	20	15	20
	0.8956	1.1798	0.8968	1.1986
	0.8875	1.1868	0.8869	1.1898
	0.8958	1.1879	0.8875	1.1896
Avg.	0.892967	1.184833	0.8904	1.192667
SD	0.004735	0.004394	0.005551	0.005139
%RSD	0.005303	0.003708	0.006234	0.004309

Ruggedness: One mL of stock solution (1000 µg/mL) was pipetted-out in to a 100 mL standard flask and diluted up to the mark with 0.1*N* NaOH to obtain 10 µg/mL. Absorbance measured at 271-275nm (Table 5).

Limit of detection and quantification: LOD and LOQ determination is based on the standard deviation of the response and slope. (LOD = 3.3 ×σ /S and LOQ = 10 ×σ /S) where σ is the standard deviation of response and S is the slope of the calibration cure.

Table 5. Ruggedness of Hydrochlorothiazide

Stat	Hydrochlorothiazide (µg/mL)	
	271 nm	275 nm
	0.5883	0,5987
	0.5765	0.565
	0.5369	0.5982
Avg.	0.567233	0.5873
SD	0.026924	0.019314
%RSD	0.047465	0.032886

3.3. Forced degradation studies

All stress decomposition studies were performed and determined by as per the test method.

Degradation studies of Hydrochlorothiazide in Acidic condition: To pipette out 1mL of stock solution

(1000µg/mL) concentration of HCT, added 1 mL of acidic medium 0.1*N* HCl was added in 10 mL of volumetric standard flask,the volume made upto the mark with 0.1*N* NaOH.The solution heated at 60°C for a period of 4 hrs. In a different time intervals the sample aliquots was withdrawn at 2 hrs and 4 hrs, then neutralized with 2 mL of 0.1*N* NaOH.For the blank, 0.5 mL solution of 0.1*N* HCl and 0.5 mL solution of 0.1*N* NaOH was used. Values are presented in table no:6.

Degradation studies of Hydrochlorothiazide in Alkaline condition: To pipette out 1 mL of the stock solution (1000µg/mL) concentration of HCT, added 1 mL of alkaline medium 0.1*N* NaOH was added in a 10 mL of volumetric standard flask, the volume made upto the mark with 0.1HCl.the solution heated at 60°c for a period of 4 hrs.in a different time intervals the sample aliquots was withdrawn at 2 and 4 hrs, and then neutralized with 2 mL of 0.1*N* HCl. For the blank, 0.5 mL solution of 0.1*N* HCl and 0.5 mL solution of 0.1*N* NaOH was used. Values are presented in table no:6.

Degradation studies of Hydrochlorothiazide in Oxidation condition: To pipette out 1 mL of the stock solution (1000µg/mL) concentration of HCT, added 1 mL of 3% v/v solution of hydrogen peroxide (oxidizing medium). Volume made up to the mark with 0.1*N* NaOH.then the solution was analyzed without heat at 0, 2 and 4 hrs, didn't find out the degradation. Further went for heated at 60°c for a period of 4 hrs.in a different time intervals the sample aliquots were withdrawn at 2 and 4 hrs. 0.1*N* NaOH used as a blank. Values are presented in table no:6.

Degradation studies of Hydrochlorothiazide in Thermal condition: HCT sample was taken in a petriplate and exposed to dry hot air oven at 0 for 2days of 1mm thickness in a petridish. 10mg of the sample was diluted with 0.1*N* NaOH in order to make the volume up to 10 mL. From this solution; dilutions were carried out to achieve the concentration for the UV-Visible analysis. Values are presented in table no:6

Degradation studies of Hydrochlorothiazide in Photo stability condition: Sample of Carvedilol was exposed to near ultraviolet lamp in photo stability chamber to exposing UV light in a petridish (1 mm thickness). In a different time intervals of 24 hrs and 48 hrs. 10mg of the sample was diluted with 0.1*N* NaOH in order to make the volume up to 10 mL. From this solution; dilutions were carried out to achieve the concentration for the UV-VIS analysis. Values are presented in table no:6

Table 6. Hydrochlorothiazide- Stress degradation studies

Stress condition	Time (hrs)	% degraded	% Recovered
0.1*N* HCl	2	11.5	88.5
	4	14.8	85.2
0.1*N* NaOH	2	12.3	87.7
	4	15.6	84.4
3% H_2O_2	2	11.7	88.3
	4	13.2	86.8
Thermal	48	0	100
Photo stability	24	15.1	84.9
	48	16.2	83.8

Conclusion

A simple, precise and accurate method was developed by UV Spectroscopy method has been developed for analysing of HCT in fixed-dose combination of formulated tablets. The method was validated for linearity, precision, accuracy, ruggedness and LOD & LOQ. The methods were found to be simple and accurate when compared to other existing methods found in literature and journal.

Acknowledgement

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