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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. In bulk and formulated dosage form. The drug was under 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 λmax of the Hydrochlorothiazide 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 5-30µg/ml for Hydrochlorothiazide respectively, 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 Hydrochlorothiazide was found to be within the limit 100% ± SD. The proposed method can be successfully applied for the method development, validation and stress degradation studies of Hydrochlorothiazide. The percentage degradation limit should be 5-20%. The drug Hydrochlorothiazide was found to be within the limit.***

Keywords: UV Spectroscopy, Hydrochlorothiazide, Stress degradation, validation

1. Introduction

Hydrochlorothiazide is chemically 6-chloro-3, 4-dihydro-2h-1, 2, 4-bezothiadiazine-7-sulphonamide-1, 1-dioxide(shown in fig no: 1).It is used in the treatment of hypertension. Hydrochlorothiazide pharmacology action, it inhibits active chloride reabsorbing at the distal tubule via the sodium chloride co-transporter, resulting in an increased in the excretion of sodium chloride and water The antihypertensive mechanism of Hydrochlorothiazide 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 Hydrochlorothiazidein forced degradation studies using UV Spectrophotometry. The specific aim of the research was to develop a UV method for the forced degradation studies of Hydrochlorothiazide, in bulk and formulated dosage form and to validate the proposed methods in accordance with ICH guidelines for the intended analytical application[4-10].

2. Materials and Methods

Instrumentation
A UV-VISIBLE spectrophotometer(LAB India) equipped withUV detector 1.0 cm matching quartz cells was used.

Chemicals and reagents
Hydrochlorothiazide was obtained from Saimirrainno PharmPvt Ltd, Chennai, Tamilnadu, India. Sodium hydroxide (AR Grade) was obtained from nice chemical (India) Pvt Ltd.

Method Development by UV Spectroscopic Method

Selection of Wavelength
The quantity containing 100 mg of Hydrochlorothiazide were taken in 100 ml standard flask, and the volume was made up to the mark with 0.1N NaOH to obtain 1000µg/ml. from which 10ml of solution was taken and diluted to 100 ml and made up to volume to obtain 100µg/ml .From the 100µg/ml solution, 10mlwas taken and transferred into 100ml standard flask, and the volume was made up to the mark with 0.1N NaOH to obtain 10 µg/ml concentration of Hydrochlorothiazide respectively. The above solution were scanned over range of 200-400nm. It is shown by fig. no: 2

3. Assay of Hydrochlorothiazide

3.1 Standard preparation

The quantity containing 100mg Hydrochlorothiazide was taken into 100ml clean dry standard flask 0.1N NaOH was added and the volume was made up to the mark to obtain 1000µg/ml and used as stock solution. Fromthe stock solution 1ml was pipetted out into a 100ml standard flask and make up to the mark with 0.1N NaOH to obtain 10µg/mlconcentration.

3.2 Sample preparation

20 tablets were weighed and powder it, a powder equivalent to 100mg of Hydrochlorothiazide was taken into a 100ml clean dry standard flask 50ml0.1N NaOH was added and sonicated the volume was make up to the mark to obtain

1000µg/mlfurther it was diluted with 0.1N sodium hydroxide to obtain 10µg/ml.

3.3 Method Validation

Linearity
The linearity of the methods is determined at six concentration levels ranging from 5-30µg/ml. The correlation co efficient of Hydrochlorothiazide was found to be 0.999 respectively.it shown in Fig no:3& table no:1.

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 Hydrochlorothiazide was found to be 101.50%, 100.44%, and 100.04% from 80%, 100% and 120% sample solutions respectively. The obtained percentage recovery of Hydrochlorothiazide was found to be within the limit. This indicates the proposed method was more accurate.It shown in table no: 2.

Precision
The Precision method was determined by performing at intraday precision andinterday precision.% RSD was found to be within the limit as per ICH guidelines. It shown in table no: 3&4.

Ruggedness
From the stock solution(1000µg/ml) to pipette out 1ml of solution in a 100ml standard flask and diluted up to the mark 0.1N NaOH to obtain 10µg/ml. Measured at 271nm to 275nm. It shown in table no: 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.

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

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

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

Degradation studies of Hydrochlorothiazide in Thermal condition:
Hydrochlorothiazide 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 0.1N NaOH in order to make the volume up to 10ml. 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 (1mm thickness). In a different time intervals of 24 hrs and 48 hrs. 10mg of the sample was diluted with 0.1N NaOH in order to make the volume up to 10ml. From this solution; dilutions were carried out to achieve the concentration for the UV-VIS analysis. Values are presented in table no: 6

4. Results and Discussion

In the present work, we have developed a newer, simple, accurate and cost effective UV Spectrophotometric method for the effective determination of Hydrochlorothiazide in bulk and formulated Tablet dosage form. The detection of λ max of Hydrochlorothiazide was found to be 273nm respectively. The percentage purity of Hydrochlorothiazide was found to be 99. 84% w/v. The calibration plot for Hydrochlorothiazide was observed to be linear in the range of 5-30µg/mL and the correlation coefficient was found to be 0.999 respectively. 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 Hydrochlorothiazide 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 Hydrochlorothiazide was found to be13.56 µg/mL respectively, LOQ of Hydrochlorothiazide was found to be 34.88µg/mL respectively.

5. Figure and Tables

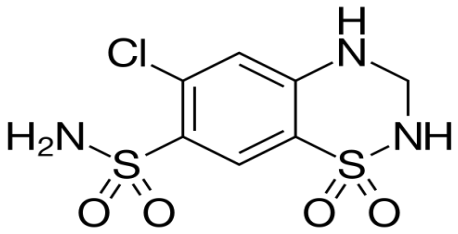

Figure 1: Structure for Hydrochlorothiazide

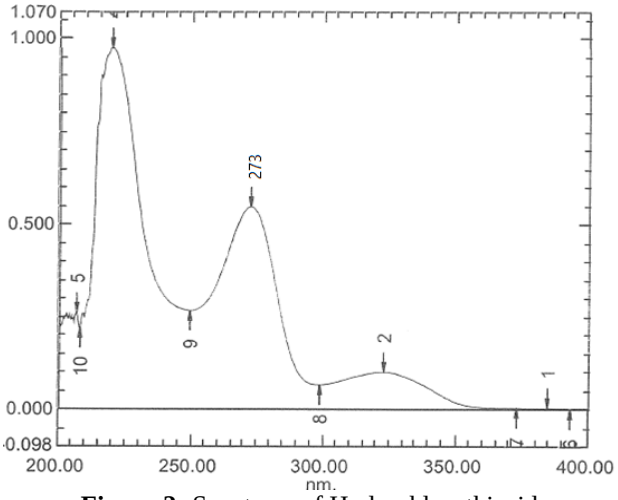

Figure 2: Spectrum of Hydrochlorothiazide

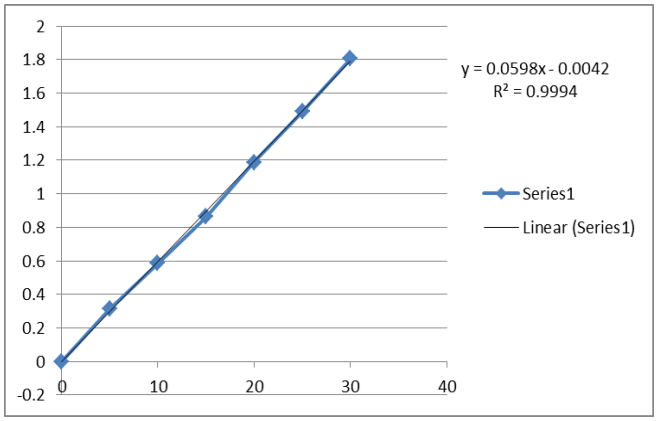

Figure 3: Calibration curve of Hydrochlorothiazide

Table 1: Result for linearity

Concentration (µg/ml)	Absorbance of Hydrochlorothiazide	Statistical analysis of Hydrochlorothiazide
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	

Table 2: Accuracy for Hydrochlorothiazide by UV method

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

Table 3: Intraday analysis of Hydrochlorothiazide by UV method

S.NO	Hydrochlorothiazide	
	15 (µg/ml)	20 (µg/ml)
	0.8999	1.1864
	0.8982	1.1698
	0.8897	1.1687
Average	0.895933	1.174967
S.D	0.005465	0.009917
%RSD	0.0069949	0.00844

Table 4: Interday analysis of Hydrochlorothiazide by UV method

S.No	Hydrochlorothiazide			
	Analysist 1		Analysist 2	
	15 (µg/ml)	20(µg/ml)	15 (µg/ml)	20 (µg/ml)
	0.8956	1.1798	0.8968	1.1986
	0.8875	1.1868	0.8869	1.1898
	0.8958	1.1879	0.8875	1.1896
Average	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

Table 5: Ruggedness of Hydrochlorothiazide

S.NO	HYDROCHLOROTHIAZIDE	
	271nm	275nm
	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

Table 6: Stress degradation studies for the Determination of Hydrochlorothiazide

S. No	Stress condition	Time	Percentage of degraded	Percentage of Recovered
1.	0.1N Hcl	2hrs	11.5	88.5
		4hrs	14.8	85.2
2.	0.1N NaOH	2hrs	12.3	87.7
		4hrs	15.6	84.4
3.	3% H_2O_2	2hrs	11.7	88.3
		4hrs	13.2	86.8
4.	Thermal	48hrs	0	100
5.	Photo stability	24hrs	15.1	84.9
		48hrs	16.2	83.8

6. Conclusion

A simple, precise and accurate method was developed by UV Spectroscopy method has been developed for analysing of Hydrochlorothiazide 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.

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