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A Stability Indicating Validated Method for the Quantitation of hydrochlorothiazide by Using Diffuse Reflectance Infrared Fourier Transform Spectroscopy in bulk and tablet dosage form

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Abstract: A quantitative method using diffuse reflectance infrared fourier transform spectroscopy (DRIFTS) was developed and validated for the estimation of hydrochlorothiazide (HCTZ) in its tablet dosage form. The solid-state samples were, prepared by dilution in dry potassium bromide and were analyzed by FTIR spectrophotometer with DRIFT sampling technique. A linear relationship for the sulphone peak area centered on 1151 cm^{-1} was observed in the range of 1-6 % w/w with good correlation coefficient of 0.999. The percent recovery of hydrochlorothiazide in marketed tablet dosage form was in the range of 100 ± 0.3071. The present reported method is precise, reproducible, and eco-friendly. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) may have a potential as an alternative method for qualitative and quantitative analysis of hydrochlorothiazide in bulk drugs and tablet dosage forms.

Keywords*:* Hydrochlorothiazide, FTIR, Validation, Tablet, Infrared spectroscopy, Stability indicating method.

Introduction:

Fourier transform infrared spectroscopy (FTIRS) is a widely explored technique in the pharmaceuticals and drug research for the identification of compounds, impurities, and determination of functional groups in qualitative analysis. Traditionally, FTIR analysis is carried out by transmission measurement technique using the transparent pallets of sample with halide salts. Quantification of some pharmaceutical agents has been reported in the literature using FTIR spectroscopy either by measuring the transmission of analyte in potassium bromide or in chloroform. Chemically hydrochlorothiazide is 6-Chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide (Figure 1)[1]. Hydrochlorothiazide is a thiazide diuretic decreases the reabsorption of electrolytes from the renal tubules by inhibiting the sodium-chloride symporter in the distal convoluted tubule (DCT) and finally decreases the osmotic gradient and water reabsorption throughout the nephron. It increases excretion of water and electrolyte together with metallic element, potassium, chloride, and metals. It has been used in the treatment of many disorders as well as swelling, hypertension, diabetes insipidus, and hyperparathyroidism[2]. In Literature survey various methods have been reported Like UV, HPLC for estimation of hydrochlorothiazide in single and in combined dosage form[3-11] but no single method is reported on FTIR for quantification of hydrochlorothiazide in bulk and tablet dosage form. Hence an attempt has been made to develop FTIR method for the estimation of hydrochlorothazide in bulk and in tablet dosage form.

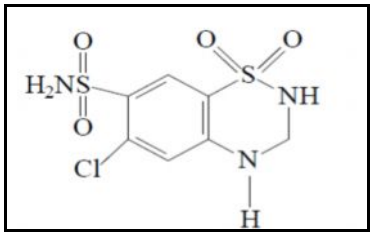

Figure 1: Chemical structure of Hydrochlorothiazide

Materials and Methods

Chemicals and Reagents

Standard sample of hydrochlorothiazide was obtained as gift sample from Ipca Laboratory Ltd., Mumbai, India. Potassium bromide (IR grade) was obtained from Merk Specialties Pvt. Ltd. Mumbai, India.

FTIR Instrumentation

The FTIR analyses were carried out on FTIR – spectrophotometer - A213747, (Shimadzu, Japan). FTIR spectra were recorded in the wavenumber range between 4000-400 cm^{-1}, averaging 45 scans per sample using a nominal resolution of 8 cm^{-1}employing background spectrum of potassium bromide. The IResolution PC software was used for collection and analysis of the data.

Preparation of working standard

Accurately weighed hydrochlorothiazide (10 mg) was mixed with 990 mg of KBr as diluent and further diluted to make a concentration of 1 % w/w of hydrochlorothiazide.

Selection of analytical wave number

Working standard (1 % w/w) of pure drug, i.e. hydrochlorothiazide was scanned in the IR range of 4000-400 cm^{-1} with resolution of 8 and 45 scans. Wave number (Intensity) parameter is selected for pure drug in such a way that one can select wave number in a range. Functional group selected for hydrochlorothiazide is O=S=O (sulphone) and wave number found in range of 1132-1159 cm^{-1}. IR spectrum of hydrochlorothiazide is shown in Figure 2.

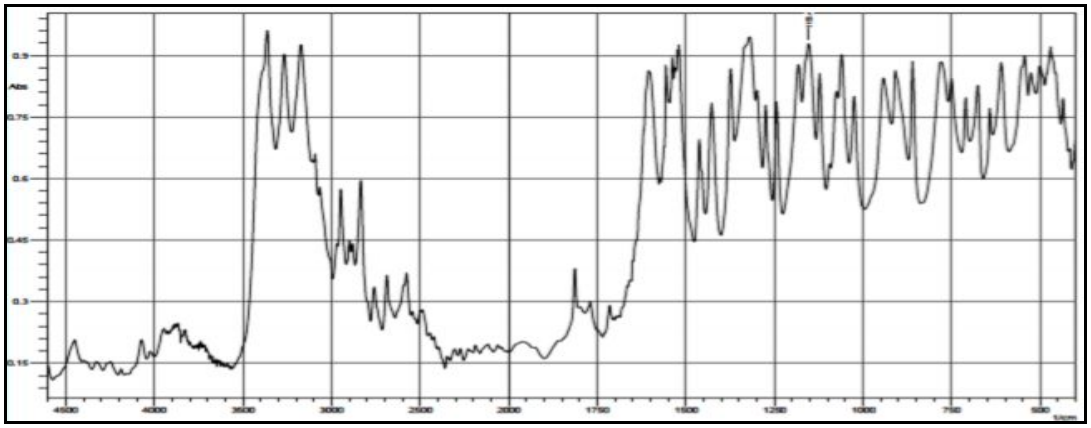

Figure 2: FT-IR spectrum of hydrochlorothiazide

Calibration Curve

Appropriate quantity of hydrochlorothiazide was diluted with potassium bromide to get around 1000 mg and triturated to ensure sample homogeneity and calibration curves were prepared for six different hydrochlorothiazide concentrations in the range of 1–6 % w/w. Each calibration standard was analyzed in the replicates of six. Area under curve (AUC) corresponding to the sulphone peak around 1132–1159 cm^{-1} was used

for the quantification and the average of six measurements was used to obtain the calibration curve. All the statistical calculations and calibration curve plotting were carried out using IResolution PC software for Windows-XP. Calibration curve is shown in Figure 3.

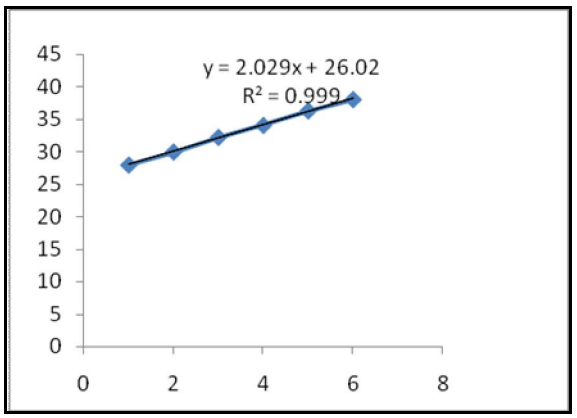

Figure 3: Calibration curve of Hydrochlorothiazide

Method Validation

The developed method was validated as per literature available and ICH guidelines[12-14] by studying the following method validation parameters.

Linearity study

Pure drug sample of hydrochlorothiazide was diluted with KBr to get concentration ranging from 1-6 % w/w. Peak area of these dilutions were measured in the range of 1132-1159 cm^{-1} using KBr as blank. Plot of peak area versus concentration were found to be linear. The linearity of proposed method was found to be in between 1-6 % w/w for hydrochlorothiazide shown in following Table 1 and Figure 3.

Table 1: Linearity study data of hydrochlorothiazide

Sr. No.	**Concentration (% w/w)**	**Peak area (1132-1159cm^{-1})**
1	1	28.01
2	2	30.01
3	3	32.25
4	4	34.093
5	5	36.328
6	6	38.321

Table 2: Optical characteristics of hydrochlorothiazide by FTIR Spectrometry

Parameters	**HCTZ**
Wave number (cm^{-1})	1151 cm^{-1}
Linearity range (% w/w)	1-6 %
Limit of detection (% w/w)	0.006
Limit of quantitation (% w/w)	0.018
Slope (m)	2.0295
Intercept (c)	26.021
Regression coefficient (R^2)	0.999

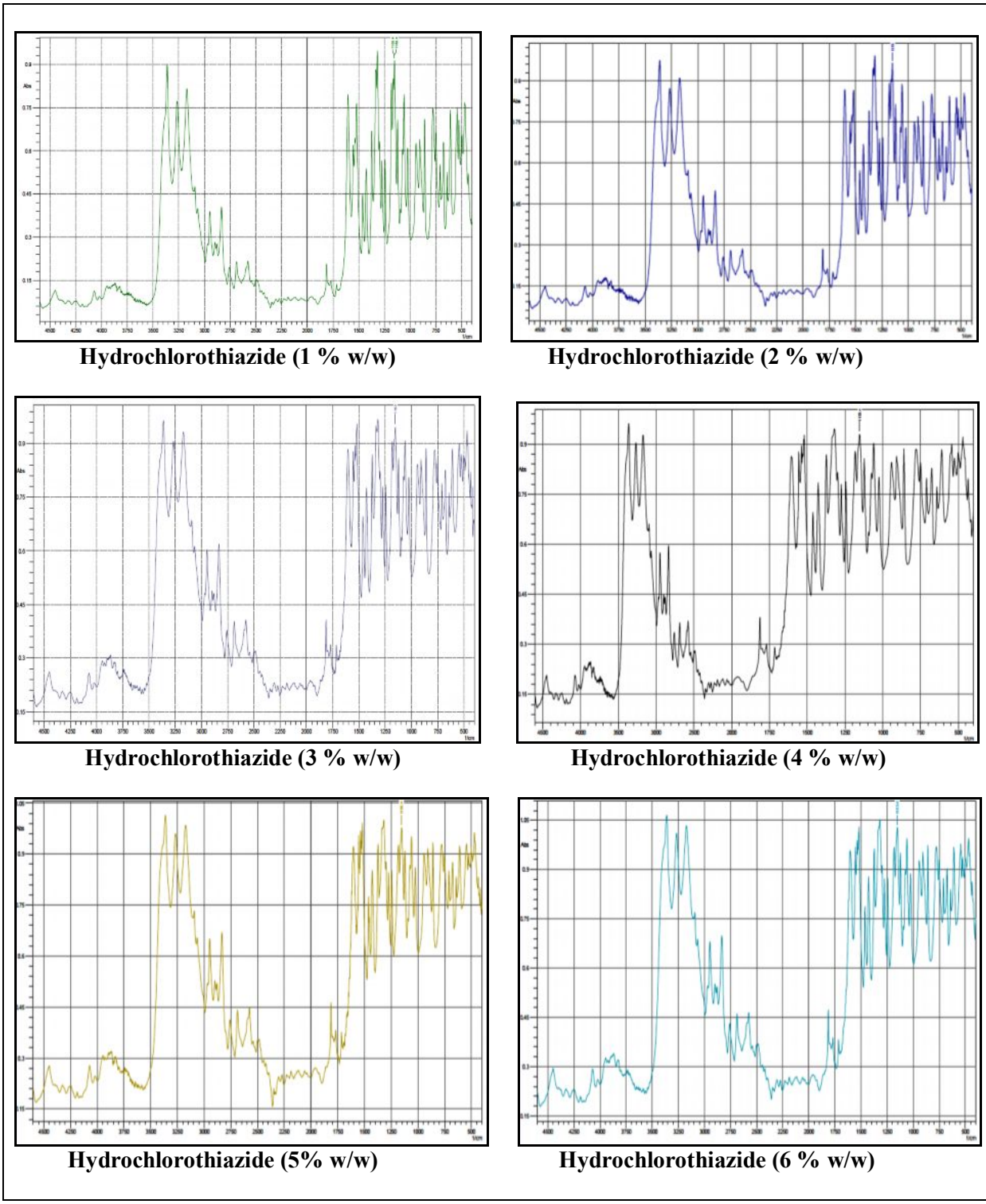

Figure 4: Linearity spectrums of hydrochlorothiazide (1-6 % w/w)

Precision

The precision of the method was evaluated by inter-day and intraday variation studies. In intraday studies, working dilutions of sample were analyzed triplicate in a day and percentage relative standard deviation (% RSD) was calculated. In the inter-day variation studies, working dilutions of sample were analyzed on three consecutive days and percentage relative standard deviation (% RSD) was calculated shown in Table 3.

Table 3: Precision data for method validation of HCTZ

Sr. No.	Interval of Time	Concentration (% w/w)	% Recovery
		HCTZ	**HCTZ**
I	Intra-day	1	99.93
II		1	100.64
III		1	100
		Mean*	99.97
		SD	0.647
		% RSD	0.648
I	Inter-day	1	99.93
II		1	100.03
III		1	99.69
		Mean*	99.42
		SD	0.655
		% RSD	0.653

** Indicates average of six determinations*

Accuracy

The accuracy of the developed method evaluated by standard addition method with recovery of pure drug at 3 different quantities (80, 100 and 120 % w/w). To the preanalysed tablet powder of hydrochlorothiazide, the known amount of hydrochlorothiazide studied. Powder corresponds to 80, 100 and 120 % w/w of label claim was added.

Recovery study was performed by 80, 100 and 120 % w/w of working standard to a preanalysed sample and the final diluted solutions prepared in such a way that their concentrations should be in linearity range and percentage amount recovered calculated accordingly. The sample was mixed thoroughly and analysed by making appropriately 1 % w/w dilutions with potassium bromide powder in three replicates. Peak area of this mixture was measured in the range of 1132-1159 cm^{-1} using KBr as blank for background.

Table 4: Recovery study data for method validation of HCTZ

Level of Recovery	Amount present (mg)	Added concentration (mg)	Amount recovered (mg)	% Recovery
	HCTZ	**HCTZ**	**HCTZ**	**HCTZ**
	12.5	10	22.46	99.83
80%	12.5	10	22.47	99.88
	12.5	10	22.56	100.2
	12.5	12.5	24.97	99.88
100%	12.5	12.5	2.12	100.5
	12.5	12.5	24.90	99.61
	12.5	15	27.33	99.41
120%	12.5	15	27.74	100.9
	12.5	15	27.41	99.68

Table 5: Statistical validation of recovery study data

Level of recovery	% Mean recovery*	SD	% RSD
	HCTZ	**HCTZ**	**HCTZ**
80%	100.1	0.241	0.243
100%	100	0.458	0.459
120%	100.09	0.791	0.793

* Indicates average of six determinations

Detection Limit and Quantitation Limit

These include visual evaluation, signal to noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 σ/S and 10 σ/S criterions respectively. Where, σ is the standard deviation of the γ-intercepts of the regression lines and S is the slope of the calibration curve.

Table 6: LOD & LOQ

Name of the drug	LOD (% w/w)	LOQ (% w/w)
HCTZ	0.006	0.018

Analysis of marketed tablet formulation

Accurately weighed 20 tablets (i.e. Aquazide) and average weight were determined. Powder weight equivalent to 12.5 mg of hydrochlorothiazide was taken and mixed with KBr and further diluted to 1 % w/w concentration. Peak of these dilutions were measured in the range of 1132-1159 cm^{-1} using KBr as blank.

Table 7: Analysis of marketed tablet formulation (Aquazide)

Sr. No.	Label claim (mg/tab)	Amount found (mg/tab)	% of Label claim
1	12.5	12.48	99.87
2	12.5	12.43	99.47
3	12.5	12.51	100.60
4	12.5	12.54	100.03
5	12.5	12.52	100.20
6	12.5	12.49	99.98
		Mean*	100
		SD	0.3072
		% RSD	0.3071

** Indicates average of six determinations*

Interaction Study of Excipients with drug:

Three tablets of marketed formulation was used and crushed to fine powder and transferred to volumetric flask. To it sufficient quantity of methanol was added and sonicate for 15 min. Then filtered and the cake was dried and scan in FTIR.

The FTIR peak of excipient is shown in Figure 5 and an overlain FTIR spectrum of pure drug and excipient is shown in Figure 6.

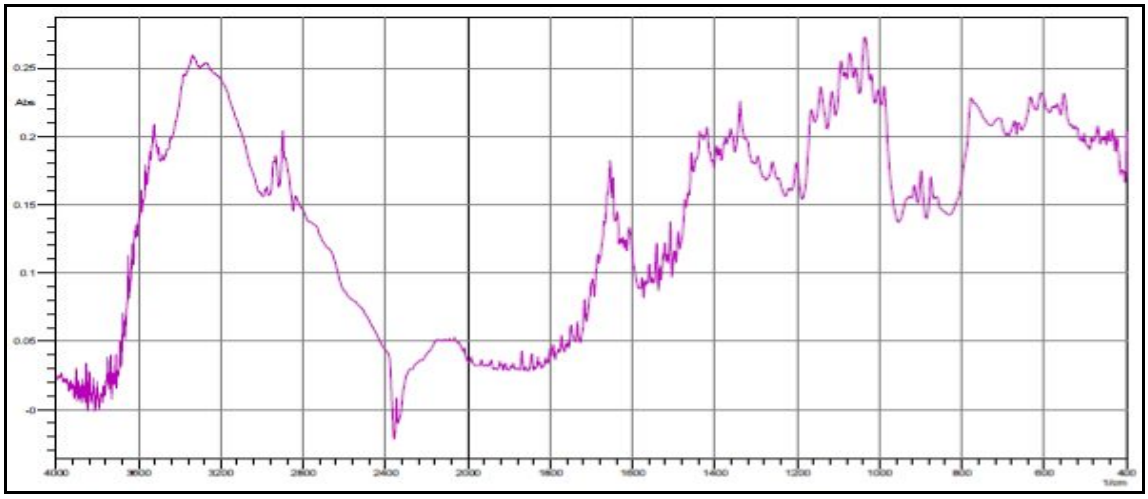

Figure 5: FTIR spectra for tablet excipient of Aquazide

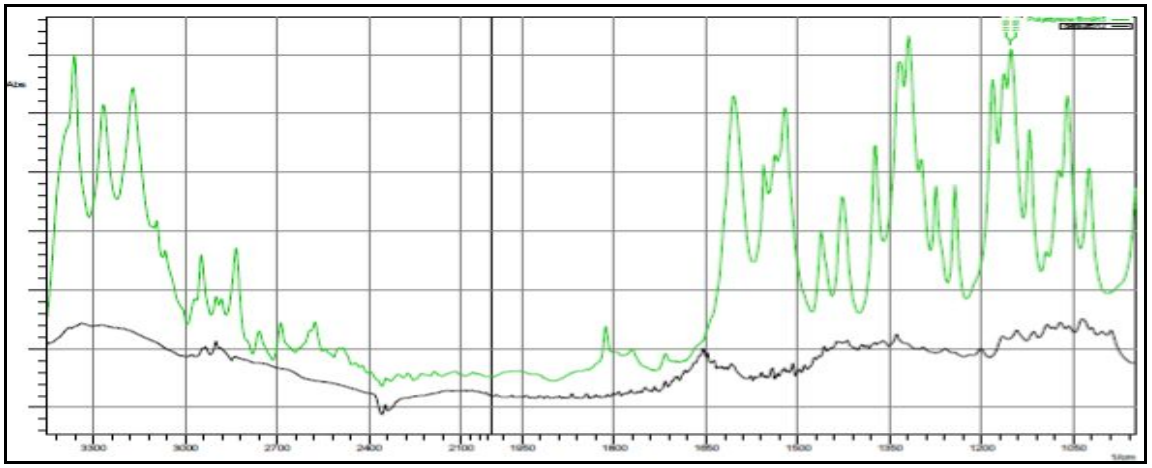

Figure 6: Overlain of FTIR spectra of tablet excipient with pure API Hydrochlorothiazide

Forced degradation study

Specificity of the method was determined by calculating percent amount of possible degradation products produced during the forced degradation study. The stress conditions applied for degradation study involved thermal (80ºC) and UV photolysis (365 nm) and degradation in sunlight.

Photolytic degradation

Pure drug hydrochlorothiazide was exposed to UV radiations for 3 h and sample was withdrawn at interval of 30 min. The sample after exposure to light was diluted with KBr to get (1% w/w). Wave number was measured in the range of 1132-1159 cm^{-1} using KBr as blank for background. Finally peak area of sample was compared with standard peak area and then percent degradation and percent assay were calculated (Figure 7).

Thermal degradation

Thermal degradation was carried out by exposing pure drug hydrochlorothiazide to dry heat at 80ºC for 3 h Sample was withdrawn at interval of 30 min. The sample after exposure to heat was diluted or mixed with KBr to get hydrochlorothiazide (1 % w/w). Wave number was measured in the range of 1132-1159 cm^{-1}. Finally peak area of sample was compared with standard peak area and then percent degradation and percent assay were calculated (Figure 8).

Degradation in Sunlight

Sunlight degradation is performed by exposing the pure drug hydrochlorothiazide to sunlight in open space for 3 h Sample was withdrawn at interval of 30 min. The sample after exposure to sunlight was diluted or mixed with KBr to get hydrochlorothiazide (1 % w/w). Wave number was measured in the range of 1132-1159 cm^{-1}. Finally peak area of sample was compared with standard peak area and percent degradation and percent assay were calculated (Figure 9).

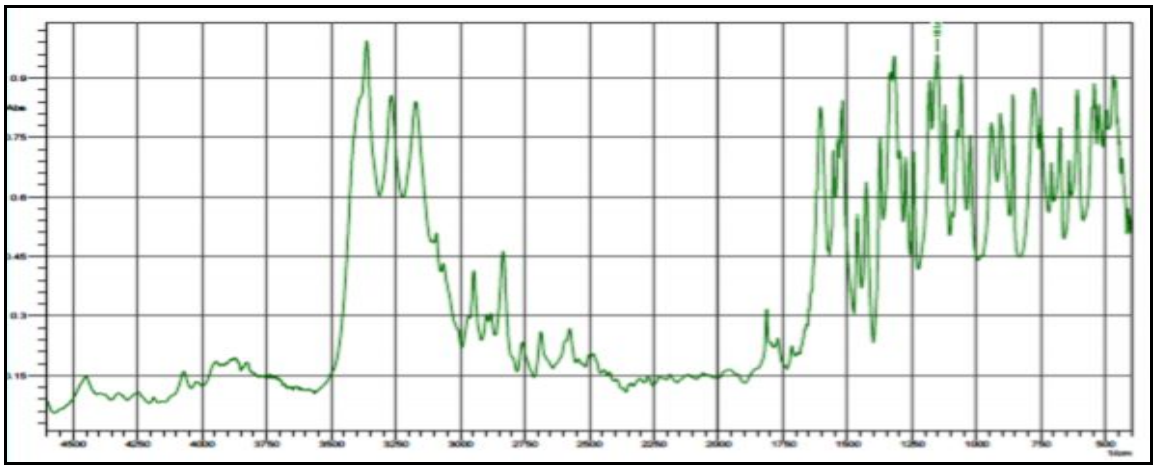

Figure 7: Photolytic degradation of hydrochlorothiazide by FTIR Spectrophotometry

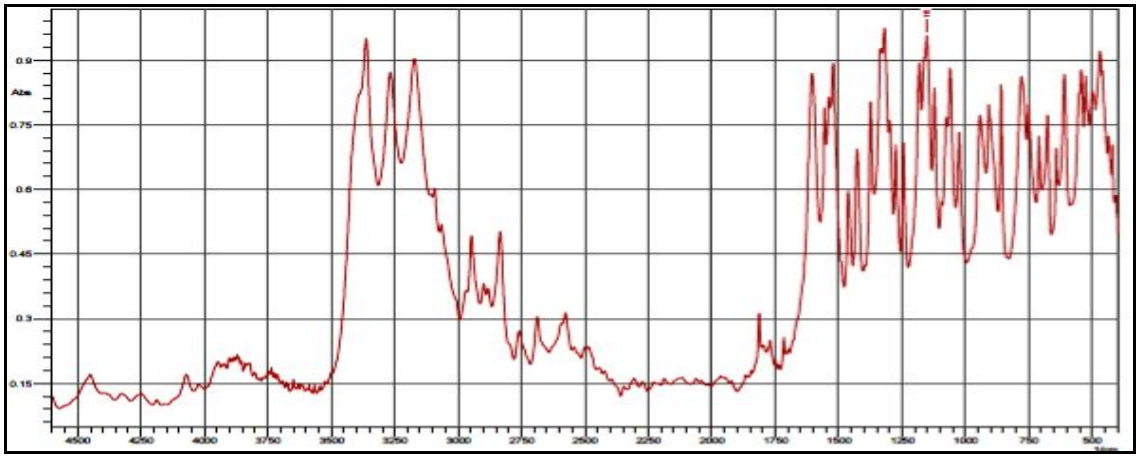

Figure 8: Thermal degradation of hydrochlorothiazide by FTIR Spectrophotometry

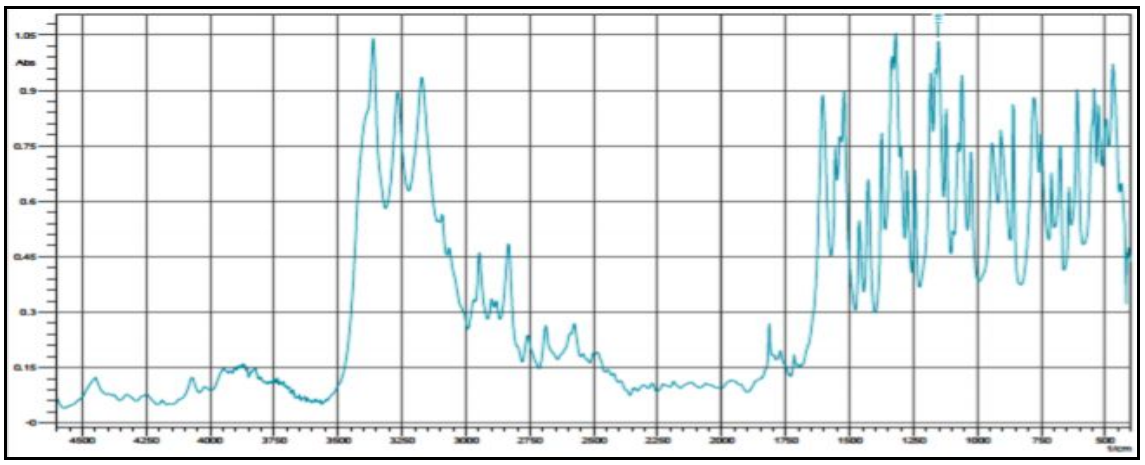

Figure 9: Sunlight degradation of hydrochlorothiazide by FTIR Spectrophotometry

Table 8: Forced degradation study data for hydrochlorothiazide by FTIR method

Sr. no.	Stress Condition	% Degradation	% Assay
		HCTZ	HCTZ
1	Photolytic degradation In UV chamber 120 min for HCTZ	20.02	79.97
2	Thermal degradation at 80^0C HCTZ for 240 min.	24.71	75.28
3	Sun Light HCTZ for 120 min.	22.24	77.75

Results and Discussion

Diffuse reflectance measurement of powdered samples typically results in relatively long path a length that increases the interaction of the infrared light with the sample. Concentrated samples may have absorbance values beyond the dynamic range of an instrument resulting in higher noise. In order to obtain the absorbance in the linear range, samples need to be diluted with non-absorbing, diffusely reflecting salts such as potassium bromide. The low-intensity absorbance bands arising from hydrochlorothiazide were not much affected by dilution in dry potassium bromide; therefore, in the present study we have used dry potassium bromide as the diluent. The most prominent absorbance band corresponding to the sulphone group centered in the range of 1132–1159 cm^{-1} for the diluted samples of hydrochlorothiazide in dry potassium bromide was within the 2.0 absorbance units. The transmittance spectra for the diluted hydrochlorothiazide samples of various concentrations, shown in Figure 2.

The area under curve (AUC) for the peak centered in the range of 1132–1159 cm^{-1} was used for the preparation of calibration curve as shown in Figure 3. The calibration curve was described by the equation $y = a + bx$. Where, y represents peak area and x represents concentration of hydrochlorothiazide. The calibration curve with good linearity was established ranging from 1 to 6 % w/w hydrochlorothiazide in potassium bromide. The corresponding linear regression equation was $y = 26.025 + 2.0295x$ and the correlation coefficient for calibration curve was 0.999 (Figure 3 and Table 1 and 2). The precision and accuracy were expressed by coefficient of variation (% RSD). The relative standard deviation (RSD) for intra-day and inter-day analysis of hydrochlorothiazide was found to be 0.648 and 0.653 respectively. The accuracy and reproducibility is evident from the data as results are close to 100 % and the value of standard deviation and % R.S.D. were found to be < 2%; shows the high precision of the method. In proposed method precision was studied as repeatability (% RSD<2) and inter and intra-day variations (% RSD<2) for drug; shows the high precision of the method (Table 3).

The accuracy of the assay method was evaluated with the recovery of pure drug from excipients at three different levels (80%, 100%, and 120%w/w of label claim) by standard addition method and the recovery data summarized in above Table 4-5. The proposed validated method was applied for the quantification of hydrochlorothiazide in tablet dosage form. The marketed tablet formulation i.e. Aquazide was analyzed using the developed method and the results of analysis are shown in Table 7. The average recovery of hydrochlorothiazide in marketed formulation was 100 %w/w of label claim and the %RSD value was 0.3071. The % recovery of label claim was in good agreement. The stress degradation studies showed that hydrochlorothiazide undergoes degradation in sunlight, photolytic and thermal condition (Table 8).

Interaction of tablet excipients was studied and found that there is no interference of excipients with drug in FTIR ranges (Figure 5 and 6).

Conclusion

Traditionally, FTIR spectroscopy is employed for the qualitative analysis of pharmaceuticals; however, with advent in sampling techniques, DRIFT spectroscopy may serve as useful technique for qualitative and quantitative analysis of solid-state pharmaceuticals. In the present paper, we report the development and validation of eco-friendly stability indicating DRIFTS method for the quantification of solid-state hydrochlorothiazide and its successful application to pharmaceuticals. The proposed method was found to be precise, accurate, and suitable for analysis of hydrochlorothiazide as bulk drug and in pharmaceutical

formulation. Thus, the developed method has the advantage of being solvent free, eco-friendly, cost effective and involving relatively simple sample preparation. The developed validated method, can be useful for the routine quality control analysis of hydrochlorothiazide in pharmaceuticals industries with desired precision and accuracy.

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References:

1. United States Pharmacopoeia 30-National formulary 25. In validation of compendial procedures, 2007; p. 1225.
2. Indian Pharmacopoeia; 2007. (2,3). 200, 133, 1034-1036, 550, 741, 902, 403-404.
3. Sachin Bhagwate and N. J. Gaikwad, Stability Indicating HPLC Method for the Determination of Hydrochlorothiazide in Pharmaceutical Dosage form, Journal of Applied Pharmaceutical Science Vol. 3 (02), February, 2013; 088-092.
4. G. Kumara Swamy, P. Sravanthi, Lakshmi M, Validated RP-HPLC method for the simultaneous determination of aliskiren, hydrochlorothiazide and amlodipine besylate in bulk and pharmaceutical formulations. *International Journal of Chem Tech Research* 2012: 4 (4); 1666-1673.
5. Rao MN, Sankar GD, Simultaneous estimation of s (-) amlodipine and hydrochlorothiazide in bulk and tablet dosage form by simultaneous equation method. *International Journal of Pharmacy and Pharmaceutical Science Research* 2011: 1(1); 1-5
6. Sahoo S, Panda PK, Mishra SK Hplc method development for simultaneous estimation of hydrochlorothiazide and perindropril in tablet dosage form. *Asian Journal of Pharmaceutical and Clinical Research* 2012; 5(2): 136-38.
7. Mahesh M, Kumanan R,Jayaveera KN, Isocratic RPHPLC-UV method development and validation for the simultaneous estimation of hydrochlorothiazide and ramiprillin tablet dosage form and bulk drug. *International Journal of Current Pharmaceutical Research* 2011; 3(2): 119-23.
8. Subramanian V, Nagappan K, Analytical method development and validation of telmisartan and hydrochlorothiazide in tablets using orthogonal polynomial function method. *International Journal of Pharmacy and Pharmaceutical Science* 2013; 5(1): 73-75.
9. **N**ikam MB, Dhamane H, Kondawar MS, Aligave A, Simultaneous estimation of valsartan, amlodipine besylate and hydrochlorothiazide by first order derivative uv spectrophotometric method. *International Journal of Pharmacy & Technology* 2010; 2(3): 642-50.
10. R Sivasakthi, Anudeepa J, Kumar SC, Rajendran SS, Development and validation of RP-HPLC and UV-spectrophotometric method for olmesartan medoxomil and hydrochlorothiazide in combined dosage form. *Der Pharmacia Lettre* 2011; 3(3): p. 478-484.
11. Belal F, Gadkariem EA, Abounassif MA, A stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms. *Journal of Pharmaceutical and Biomedical Analysis 2001*; 24(3): 335–342.
12. Monika Bakshi, Saranjit Singh, Development of validated stability-indicating assay methods-critical review. *Journal of Pharmaceutical and Biomedical Analysis,* 2002; 1011-1040.
13. ICH, Stability testing of new drug substances and products Q1A (R2), international conference on harmonization, IFPMA, Geneva, 2003.
14. ICH, Q2 (R1), "Validation of analytical procedures: text and methodology" In proceedings the international conference on harmonization; IFPMA, Geneva, 2005.

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