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A Validated FTIR Method for the Quantification of Clonidine Hydrochloride in Bulk and Tablet Formulation

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Abstract: A new simple, precise, accurate, reproducible and eco-friendly, validated analytical method for the quantification of Clonidine hydrochloride (Cl. Hcl) in tablet formulation using diffuse reflectance infrared fourier transform spectroscopy (FT-IR) was developed. Solid-state samples were prepared by dilutions in dry potassium bromide (KBr). The wave number 1658 cm^{-1} was selected for Clonidine hydrochloride. Linearity study carried out in concentration range of 0.1-0.6 % w/w for Clonidine hydrochloride with the correlation coefficient (R^2) of 0.997 and % RSD was found to be less than 2. The proposed method was validated for accuracy, precision, linearity, limits of detection (LOD) and quantitation (LOQ) and reproducibility. Accuracy and precision shows good compliance with ICH guidelines. The percentage recovery for Clonidine hydrochloride in marketed dosage form was in the range of 99.93-100.70 %. The developed FT-IR method can be used for the estimation of drug in bulk and in marketed dosage form.

Keywords: Clonidine hydrochloride, FT-IR spectroscopy, KBr.

Introduction:

Chemically Clonidine is 2-(2, 6-Dichloroanilino)-2 imidazoline hydrochloride [1, 2] (Figure 1), It is used as centrally actingα2 agonist drug which inhibits stimulation of the sympathetic nervous system. This causes dilation of the peripheral blood vessels or decreased cardiac output & thereby, reducing blood pressure[3]. In Literature survey various methods have been reported Like UV, HPLC for estimation of Clonidine in single and in combined dosage form[4-7] but no single method is reported on FTIR for quantification of Clonidine in bulk and tablet dosage form. Hence an attempt has been made to develop FTIR method for the estimation of Clonidine in bulk and in tablet dosage form.

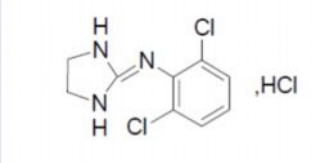

Figure 1: Structure of Clonidine HCl

Materials and Methods

2.1 Apparatus and Instruments

The FT-IR analysis was carried out on (SHIMADZU IR AFFINITY-1) spectrophotometer Serial no-A213747 attached with computer operated software IR solution with DRS detector. Analytical weighing balance (AA-2200) and hot air oven were used during the study.

2.2 Chemicals and Reagents

Standard sample of Clonidine hydrochloride was obtained as a gift samples from Neon Lab. Ltd., Mumbai. Marketed formulation, Arkamin (clonidine hydrochloride 0.1mg) of Unichem Pharma Limited was purchased from market. KBr (IR Grade) was obtained from MERCK, Mumbai.

2.3 Solvent selection:

Solid sampling method was used for FT-IR method development and KBr was selected as diluent as, it is transparent to IR- radiation and its peaks does not interfere with peaks of drug.

Selection of analytical wave number

Working standard (0.1 % w/w) of pure drug, i.e. Clonidine hydrochloride was scanned in the IR range of 4000-400 cm^{-1} with resolution of 8 and 45 scans. Wave number (Intensity) parameter was selected for pure drug in such a way that one can select wave number in a range. Functional group selected for Clonidine hydrochloride was C=N (emine) and wave number found in range of 1629-1691 cm^{-1}. IR spectrum of clonidine hydrochloride is shown in Figure 2.

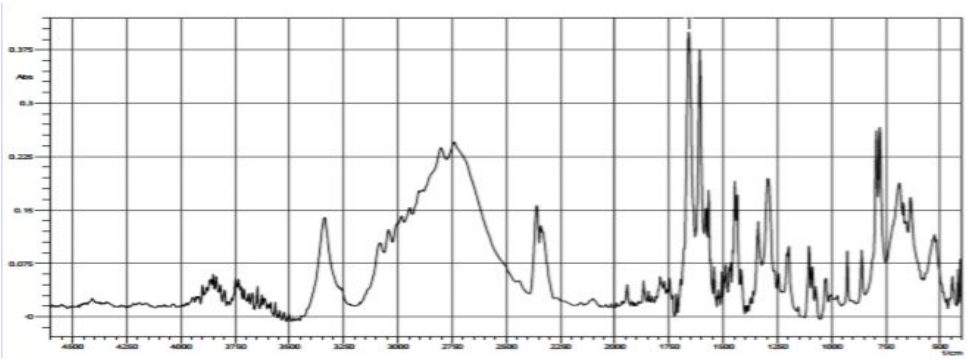

Figure 2: FT-IR spectrum of Clonidine hydrochloride

Calibration Curve

Calibration curves were prepared for six different concentrations in the range of 0.1–0.6 % w/w. Appropriate quantity of clonidine hydrochloride was diluted with potassium bromide to get around 1000 mg and triturated to ensure sample homogeneity. Area under curve (AUC) corresponding to the C=N peak around 1629-1691cm^{-1} was used for the quantification and the average of six measurements was used to obtain the calibration curve. The results are shown in Figure 3 and 4.

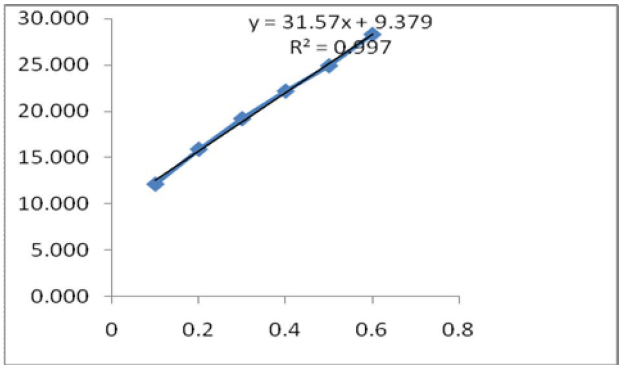

Figure 3: Calibration curve of Clonidine hydrochloride

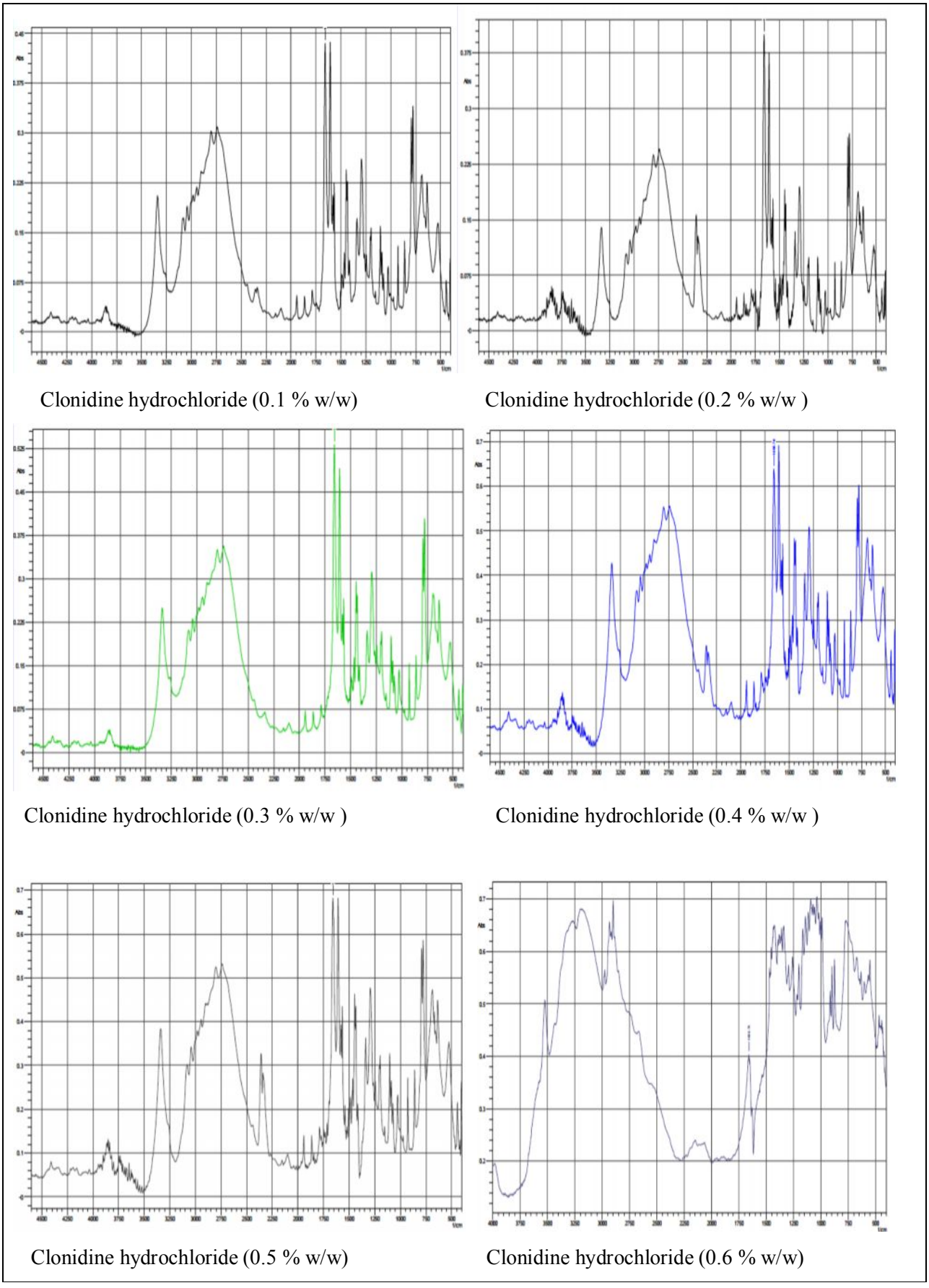

Figure 4: Linearity spectrum of clonidine hydrochloride (0.1- 0.6 % w/w)

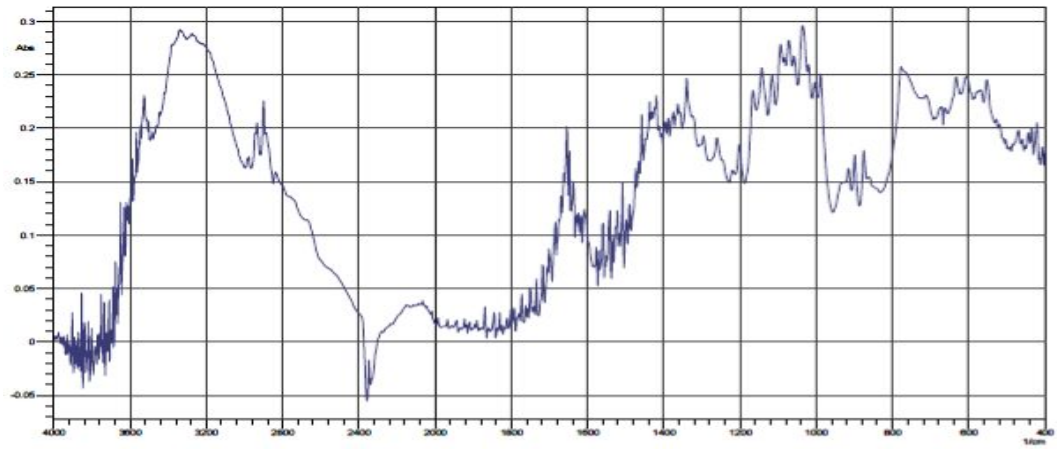

Figure 5: FTIR spectra for Arkamin tablet excipient (0.1mg label claim)

Interaction of Excipients with drug:

Three tablets of marketed formulation was taken and crushed to fine powder and transferred to conical flask and to it sufficient quantity of methanol was added and sonicated for 15 min then filtered. The cake was dried and scanned in FTIR range of 4000-400 cm^{-1} with resolution of 8 and 45 scans and spectra was recorded.

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.

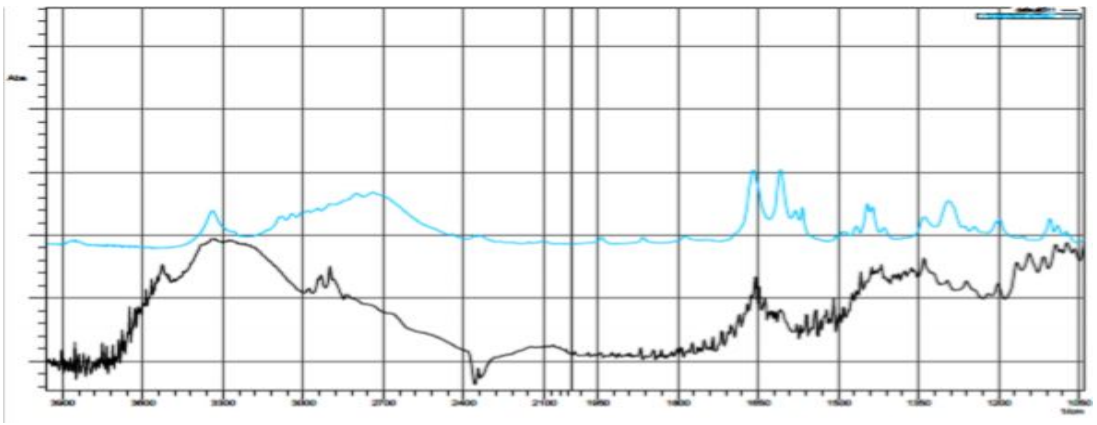

Figure 6: Overlain FTIR spectra of tablet excipient with pure clonidine hydrochloride

3. Method Validation[8-10]

The proposed method was validated for accuracy, precision, linearity, limits of detection (LOD) and quantitation (LOQ) and reproducibility. The method validation was performed as per ICH guidelines [ICH Q2 (R1)2005].

3.1 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. The precision data were mentioned in Table 1.

Table 1: Precision data for method validation of Cl. HCl

Sr. No.	Interval of Time	Concentration (% w/w)	% Recovery
		Cl. Hcl	**Cl. Hcl**
I	Intra-day	0.1	100.87
II		0.1	99.09
III		0.1	100.02
		Mean*	100
		SD	0.884
		% RSD	0.885
I	Inter-day	0.1	100.53
II		0.1	98.96
III		0.1	100.49
		Mean*	100
		SD	0.899
		% RSD	0.899

* Indicates average of six determinations

3.2 Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%) as per ICH guidelines.

As per the label claim, tablet contains 0.1 mg of clonidine hydrochloride and to analyze the sample standard addition method was adopted and 0.9 mg of standard pure clonidine hydrochloride was added and different levels of the standard concentration according to 80%, 100% and 120% were made and % mean recoveries was calculated. The results of recovery study are reported in Table 2 and 3.

3.3 Linearity

The linearity study was performed by preparing standard dilution of clonidine hydrochloride range from 0.1 to 0.6 % w/w. The calibration graph was plotted for each concentration Vs peak area of wave number for clonidine hydrochloride separately. The results are shown in Table 4.

3.4 LOD and LOQ

ICH guideline describes several approaches to determine the detection and quantitation limits. 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 calculated by use of the following equations

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where, ó is the standard deviation of the peak areas of the drugs, taken as a measure of noise, and S is the slope of the corresponding calibration curve. The values are mentioned in Table 5.

4.0 Analysis of marketed tablet formulation

Twenty tablets of marketed formulation were weighed; average weight was determined and crushed to fine powder. Appropriate quantity of powder was diluted to get around 1000 mg sample contains 0.1 % w/w of

clonidine hydrochloride. The solid sample was homogeneously mixed by triturating. The analysis was carried out using same samples in six replicates and the results are mentioned in Table 6.

Table 2: Recovery study data

Level of Recovery	Amount present (mg)	Added concentration (mg)	Amount recovered (mg)	% Recovery
	Cl. Hcl	Cl. Hcl	Cl. Hcl	Cl. Hcl
80%	1	0.8	0.80	100.83
	1	0.8	0.79	99.36
	1	0.8	0.8	99.79
100%	1	1	0.99	99.69
	1	1	0.99	99.73
	1	1	1.05	100.56
120%	1	1.2	1.2	100.46
	1	1.2	1.19	99.44
	1	1.2	1.20	100.08

Table 3: **Statistical validation of recovery study data**

Level of Recovery	% Mean Recovery *	SD*	% RSD*
	Cl. Hcl	Cl. Hcl	Cl. Hcl
80%	100.09	0.752	0.754
100%	100.03	0.490	0.492
120%	100.00	0.143	0.143

* Indicates average of three determinations

Table 4: Linear regression data for calibration curve of Cl. HCl

Name of the drug	Linearity range (% w/w)	R^2	Slope	Intercept
Cl. Hcl	0.1-0.6	0.997	31.57	9.379

Table 5: LOD & LOQ

Name of the drug	LOD (%w/w)	LOQ (% w/w)
Clonidine hydrochloride	0.0006	0.018

Table 6: Analysis of tablet formulation

Sr. No	Label claim (mg/tab)	Amount found (mg/tab)	% of Label claim
	Cl. Hcl	Cl. Hcl	Cl. Hcl
1	0.1	0.107	100.7
2	0.1	0.098	98.97
3	0.1	0.105	100.7
4	0.1	0.098	98.93
5	0.1	0.100	100
6	0.1	0.099	99.9

5.0 Force degradation studies

Force degradation study, was performed on clonidine hydrochloride to prove the stability indicating property of the method. The stress conditions applied for degradation study involved thermal, photolytic and sunlight degradation [ICH Q1A (R2) 2003].

Photolytic degradation

Pure drug was exposed to UV radiations and samples withdrawn at interval of 30 min. The samples after exposure to light were diluted with KBr to get concentration 0.1 % w/w and scanned in FTIR range of

4000-400 cm^{-1} with resolution of 8 and 45 scans. The FTIR spectrum of photolytic degradation is shown in Figure 7.

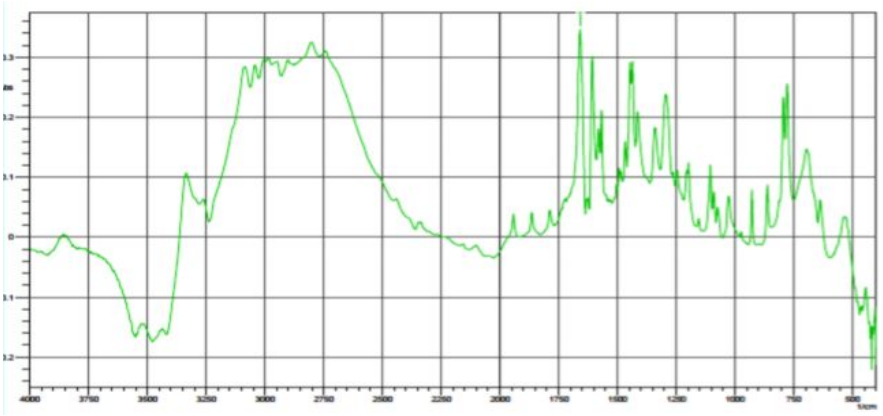

Figure 7: FTIR of Photolytic degradation of clonidine hydrochloride after 120

Thermal degradation

Thermal degradation was carried out by exposing pure drugs to dry heat at 80ºC. Samples were withdrawn at interval of 30 min. The samples after exposure to heat were diluted or mixed with KBr to get concentration 0.1 % w/w. and scanned in FTIR range of 4000-400 cm^{-1} with resolution of 8 and 45 scans. The FTIR spectrum of thermal degradation is shown in Figure 8.

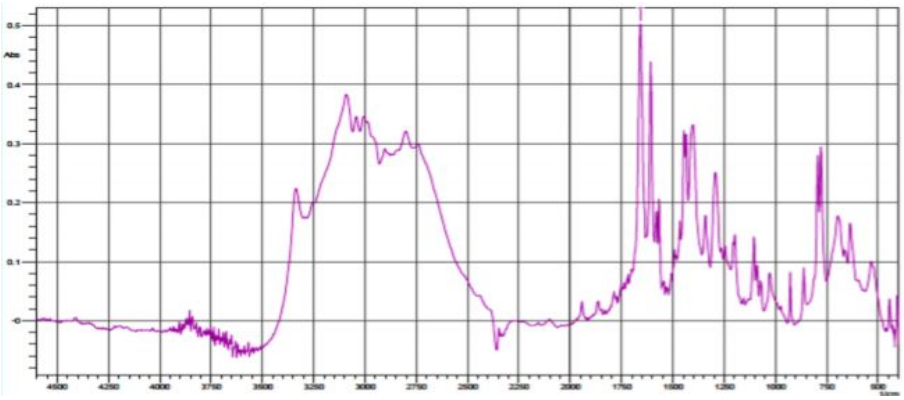

Figure 8: FTIR of Thermal degradation of clonidine hydrochloride after240 min.

Degradation in Sunlight

Sunlight degradation was performed by exposing the pure drugs to sunlight in open space. Samples are withdrawn at interval of 30 min. The samples after exposure to sunlight were diluted or mixed with KBr to get concentration 0.1 % w/w and scanned in FTIR range of 4000-400 cm^{-1} with resolution of 8 and 45 scans. The FTIR spectrum of thermal degradation is shown in Figure 9.

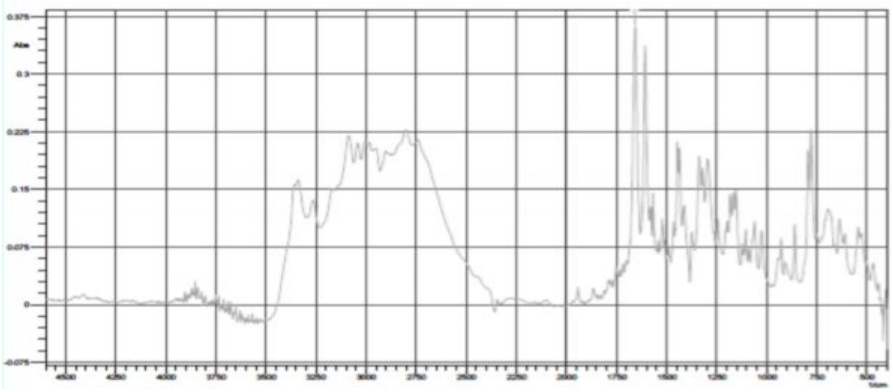

Figure 9: FTIR of Sunlight degradation of clonidine hydrochloride after240 min.

Table 7: Statistical validation: analysis of tablet formulation

Name of the drug	Mean*	SD*	% RSD*
Cl. Hcl	99.93	0.8791	0.8792

* Indicates average of six determinations

Table 8: Results of forced degradation

Sr. No.	Condition	% Degradation	% Assay
		Cl. HCL	Cl. HCL
1	Photolytic degradation In UV chamber (120 min) for Clonidine hydrochloride.	16	84
2	Thermal degradation at 80^0 (240 min.) for Clonidine hydrochloride	24.93	75.07
3	Sun Light degradation (120 min.) Clonidine hydrochloride	19.50	80.5

Table 9: Summary of validation parameters

Parameters		SAL
Linearity range (µg/ml)		0.1–0.6
Correlation coefficient (R^2)		0.997
Precision (%RSD)	Intra–day	0.885
	Inter–day	0.889
	80% ± %RSD	100.09±0.754
Accuracy (%)	100% ± %RSD	100.03±0.492
	120% ± %RSD	100.00±0.153
LOD %w/w		0.0006
LOQ %w/w		0.018

6. Results and Discussion

Diffuse reflectance measurement of powdered samples typically results in relatively long pathlengths 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 nonabsorbing, diffusely reflecting salts such as potassium bromide. The low intensity absorbance bands arising from Clonidine hydrochloride 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 C=N group centered in the range of 1629 – 1692 cm^{-1} for the diluted samples of Clonidine hydrochloride in dry potassium bromide was within the 2.0 absorbance units. The transmittance spectra for the diluted Clonidine hydrochloride samples of various concentrations are shown in Figure 3.

The area under curve (AUC) for the peak centered in the range of 1629 – 1692 cm^{-1} was used for the preparation of calibration curve as shown in Figure 2. The calibration curve is described by the equation $y = a + bx$, where y represents peak area and x represents concentration of Clonidine hydrochloride. The calibration curve with good linearity was established ranging from 0.1 to 0.6 % w/w Clonidine hydrochloride in potassium bromide. The corresponding linear regression equation was $y = 9.379 + 31.579x$ and the correlation coefficient for calibration curve were 0.997 (Figure 2 and Table 1, 2). The precision and accuracy was expressed by coefficient of variation (% RSD). The relative standard deviation (RSD) for intraday and interday analysis of clonidine hydrochloride was found to be 0.885 and 0.889 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 intraday variations (% RSD < 2) for both drugs; 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 3 different levels (80%, 100%, and 120% w/w of label claim) by standard addition method and the recovery data is summarized in Table 2 and 3. The proposed validated method was applied for the quantification of Clonidine hydrochloride in tablet dosage form. The marketed tablet formulation i.e. Arkamin was analyzed using the developed method and the results of analysis are shown in Table 6. The average recovery of Clonidine hydrochloride in marketed formulation was 99.93% w/w of label claim and the % RSD value was 0.879. The % recovery of label claim was in good agreement. The stress degradation studies showed that Clonidine hydrochloride 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 Clonidine hydrochloride and its successful application to pharmaceuticals. The proposed method was found to be precise, accurate, and suitable for analysis of Clonidine hydrochloride as bulk drug and in pharmaceutical formulation. Thus, the developed method has the advantage of being solvent free, eco-friendly, and cost effective and involving relatively simple sample preparation. The developed validated method can be useful for the routine quality control analysis of Clonidine hydrochloride in pharmaceuticals industries with desired precision and accuracy.

7. Acknowledgements

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