Development and Validation of Novel FTIR Method for Quantitative Estimation of Silodosin in Bulk and Pharmaceutical Dosage Forms

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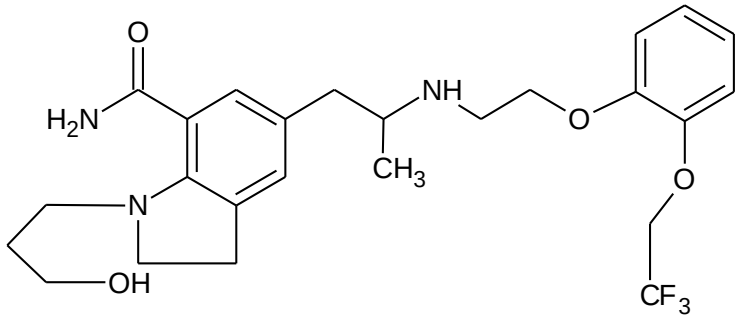
ABSTRACT
Silodosin was quantified in both pure and pharmaceutical dosage forms using a novel Fourier transform infrared (FTIR) spectroscopic technique. Silodosin is an alpha-blocker that works by relaxing the bladder and prostate muscles to alleviate benign prostatic hyperplasia symptoms. After baseline correction, the infrared spectrum revealed a variety of peaks, including intense, distinct peaks at 1620 cm^{-1} (C=O), 3201.61 cm^{-1} (NH), and 3384.84 cm^{-1} (OH). The method met the majority of validation requirements in the 40–200 µg/mg range, with a coefficient of determination of 0.998, obtained using a simple calibration model. While FTIR methods are less complex, less expensive, and faster than other methods, they can be used to estimate silodosin in a variety of dose forms.

Key words: *FTIR, Infrared spectrum, baseline correction, validation.*

INTRODUCTION
Silodosin [1] is an alpha-1 adrenergic receptor antagonist used to treat BPH symptoms. Benign prostatic hyperplasia is a benign enlargement of the prostate gland that causes lower urinary symptoms and reduces patients' quality of life. Silodosin works by relaxing the lower urinary tract and relieving urinary symptoms and bladder outlet obstruction [2].
The IUPAC name of Silodosin is 1-(3-hydroxypropyl)-5-[(2R)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl}amino)propyl]-2,3-dihydro-1H-indole-7-carboxamide. Its molecular formula and molecular weight were found to be $C_{25}H_{32}F_3N_3O_4$ and 495.5345 respectively. Its structural formula is as follows (Figure.1).



Silodosin

Figure 1. Structure of silodosin

According to a literature review [3-10], methods for estimating Silodosin include HPLC, UV spectrophotometric, and UPLC. Due to the lack of an FTIR method for silodosin estimation, As a result, the current research is an attempt to use FTIR to develop a novel, sensitive method for quantifying silodosin. When compared to HPLC analysis, FTIR analysis [11-14] can be used for quantitative estimates because it takes less time to analyse a sample. When compared to UV-visible light, IR radiation can penetrate deeper layers of the medication. As a result, FTIR may identify that tiny amount of drug as well.

EXPERIMENTAL

Apparatus

An FTIR (Fourier transform infrared) spectrophotometer of the Shimadzu 8400S type is used to obtain the infra-red spectrum. FTIR spectrums were recorded in the wave number range of 4000-400 cm^{-1}, with a nominal resolution of 4 cm^{-1}, with DLATGS used as a detector for FTIR. IR Solutions software was used to collect and analyse the data.

Reagents

Potassium bromide of IR grade is obtained from SD Fine Chemicals Limited (SDFCL), Mumbai, and Methanol of HPLC grade is obtained from Merck Specialities Private Limited. Silodosin pure form is obtained as a free gift sample from Ero Labs, Hyderabad.

Standard stocks of silodosin KBr

To accurately weigh 100 mg of silodosin, 100 mg of dried KBr was mixed with geometric mixing to form a stock of 1000 µg/mg. Mixing should be properly done so that each pellet contains a uniformly distributed drug.

Preparation of working standard

From the stock (1000 µg/mg of silodosin), 2, 4, 6, 8, 10 mg of silodosin and KBr were weighed accurately and diluted to 50 mg with dried KBr to make a final concentration of 40, 80, 120, 160, 200 µg/mg of silodosin, respectively. Mixing of the drug and dried KBr was done properly for uniform mixing.

Calibration curve

Six distinct silodosin standard concentrations in the range of 40–200 g/mg were used to create a calibration curve. To get each concentration, an adequate amount of silodosin was diluted with potassium bromide and triturated to assure sample homogeneity. In the experimental concentration range of 40–200μg/mg, the drug's response was shown to be linear. With an R2 value of 0.9995 and a regression equation of y= 0.0082x + 0.2601, the calibration curve was determined to be linear. The O-H stretching, N-H stretching, alkane bending, C=O stretching, and C=C bending peaks in the IR spectra of Silodosin are 3384 cm^{-1}, 3201.61 cm^{-1}, 2941.24 cm^{-1}, 1630 cm^{-1}, and 1508 cm^{-1}, respectively. For quantitative investigation of silodosin, the 1630 cm-1 group was selected because it showed a prominent, strong peak that increased linearly as the concentration increased. The value of the coefficient correlation should not be less than 0.999. Within the defined concentration range of 40–200μg/mg for silodosin, the drug's response was determined to be linear, with a coefficient correlation of 0.9995.

Validation of the method

The developed FTIR method was validated by specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy [15].

Linearity

Each of the working standards of silodosin (40,80,120, 160, 200 μg/mg) was produced and evaluated in FTIR. For the standard solutions, the absorbance of the peaks at 1635.52 cm^{-1} was measured. Standard calibration curves were plotted between concentration and absorbance. Regression analysis was used to determine linearity; the regression equation and coefficient of determination were provided.

Limit of Detection and Limit of Quantitation

Limit of detection (LOD) and limit of quantification (LOQ) was assessed by calculation from the regression curve. LOD and LOQ was calculated by formula

$$\mathbf{LOD=3.3\sigma/S(1.5)}$$
$$\mathbf{LOQ=10\sigma/S(1.6)}$$

Where σ = the standard deviation of the response
S = the slope of the calibration curve

Sandell's sensitivity

The lowest concentration of silodosin (40μg/mg). Calculate the Sandell's sensitivity using the following formula.

Sandell's sensitivity (JJ) = Concentration(μg/100mg)×0.001/absorbance value

Precision

Repeatability and intermediate precision were used to evaluate the method's precision. The repeatability was tested by extensively examining silodosin standard at 100% w/w six times on the same day. Repeated experiments were used to assess the method's inter-day accuracy (three different days).

Accuracy

For drug

The % recovery of silodosin was calculated using the conventional addition technique at three different concentrations (80, 100, and 120 %). A known quantity of silodosin was added to the tablet sample preparation. The percent recovery was estimated by measuring absorbance and fitting these values into the calibration curve's regression equation. At each level, the percent relative standard deviation (RSD) was determined. The acquired findings are presented in the table.

Assay of Silodosin tablets

After calculating their average weight, twenty tablets (SILODAL 8 MG) were triturated. The equivalent of one tablet powder was then transferred to an Eppendorf tube and dissolved in methanol. It was vortexed for 2 minutes before being centrifuged for 10 minutes at 5000rpm. The supernatant that resulted was then collected and evaporated overnight. The leftovers were gathered. After that, the whole residue was triturated with 50mg of KBr to produce a 120 g/mg
pellet, which was scanned in absorbance mode. The following formula is used to compute the quantity of two medicines included in the tablet.

$$\text{Assay} = \frac{\text{Concentration } \mu g/mg \text{ X Dilution factor X Average weight of the tablet (mg)}}{\text{Weight of the tablet powder taken (mg) X Label claim of the drug}} * 100$$

RESULTS AND DISCUSSION

In the experimental concentration range of 40-200 μg/mg, the drug's response was shown to be linear. With an R^2 value of 0.9995 and a regression equation of y= 0.0082x + 0.2601, the calibration curve was determined to be linear. For these investigations, the resulting R^2value was found appropriate for demonstrating the strategy's linearity.(Table.1)
The value of the coefficient correlation should not be less than 0.999. Within the defined concentration range of 40-200g/mg for silodosin, the response of the drug was found to be linear, with a coefficient correlation of 0.9995. (Figure.2)

Table 1 Standard calibration curve data for silodosin

S.No	Concentration(µg/mg)	Absorbance*at1635cm^{-1}
1	40	0.584
2	80	0.927
3	120	1.227
4	160	1.58
5	200	1.895

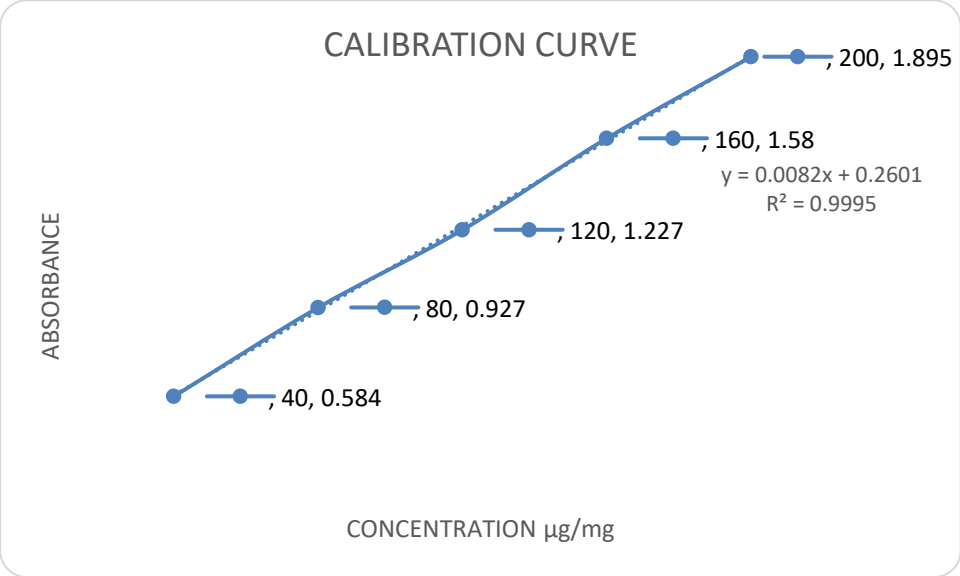


Figure 2 Standard calibration curve of silodosin

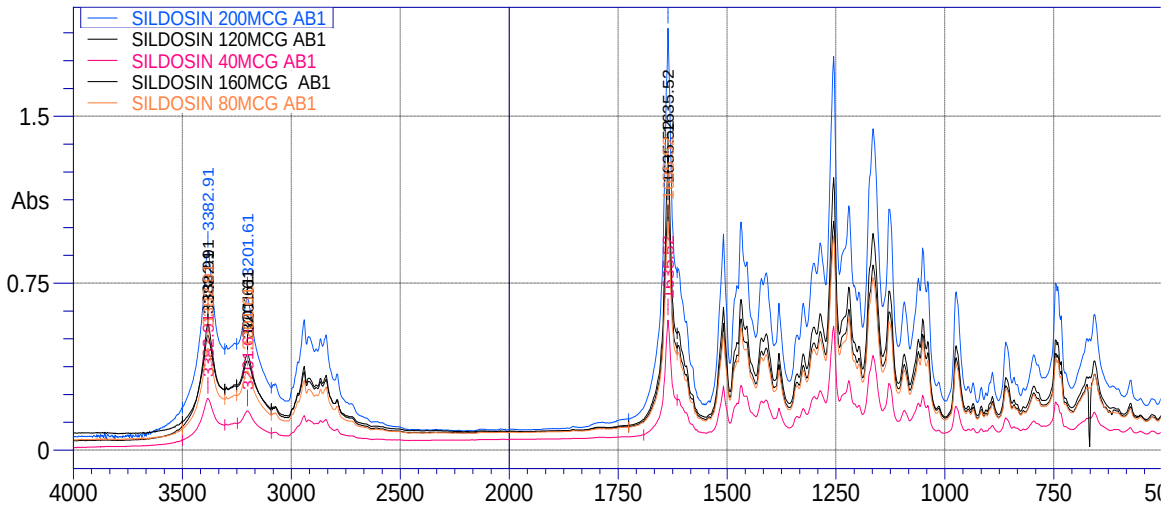


Figure 3 Overlay of Linearity spectrum of Silodosin

Silodosin's LOD and LOQ were determined to be 23.85 µg/mg and 72.28 µg/mg, respectively. This reveals the strategy's sensitivity. The lowest concentration of silodosin (40g/mg) yielded a sensitivity of 0.068 g/cm^2 according to Sandell's data. In terms of repeatability and precision, the precision of the developed analytical technique was reported. The following table.2 shows the repeatability findings for six replicates of standard silodosin pellets. The percentage RSD readings were found to be within the limits. As a result, the approach that was devised was exact.

Table 2. Repeatability data of silodosin

S.No	Concentration(µg/mg)	Absorbance	Mean ±SD	%RSD
1	120	1.189	1.214 ± 0.013	1.09%
2	120	1.221		
3	120	1.224		
4	120	1.224		
5	120	1.218		
6	120	1.212		

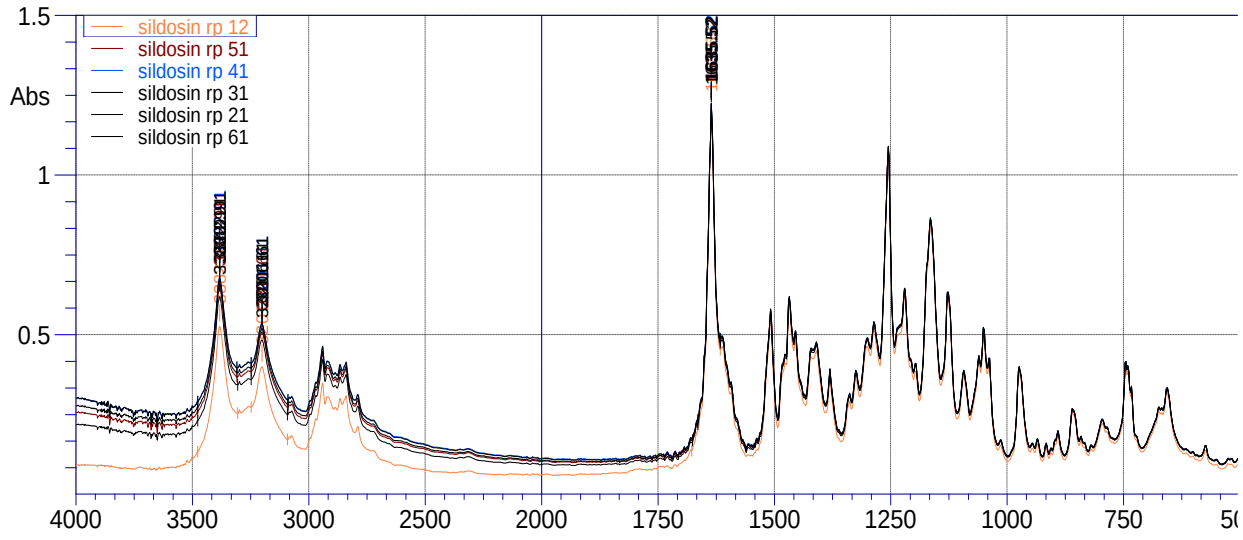


Figure 4: Over lay of Repeatability spectra of Silodosin

The following table.3 show the findings of three duplicates of each concentration of silodosin (96 µg/mg, 120 µg/mg, and 144 µg/mg). The % of RSD values were within the limits, according to the findings. As a result, the procedure created was precise.

Table 3. Interday precision data of silodosin

S.No	Concentration µg/mg	Absorbance		Mean*± SD	%RSD
1	96	Day 1	1.72	1.75 ± 0.03	1.7%
		Day 2	1.78		
		Day 3	1.76		
2	120	Day 1	1.92	1.87 ± 0.037	1.6%
		Day 2	1.85		
		Day 3	1.86		
3	144	Day 1	1.93	1.94 ± 0.015	0.5%
		Day 2	1.94		
		Day 3	1.96		

By using the standard addition technique to calculate the % recovery of silodosin, an accuracy study was conducted. By measuring the absorption and fitting these values into the calibration curve's y on x regression, the recovery % was determined. The following table.4 displays the findings obtained for silodosin recovery data.

Table 4. Recovery data for silodosin drug product

Spike level	Absorbance	Concentration recovered	% Recovery
80%	1.16	216	111.5
100%	1.28	240	118.4
120%	1.58	264	91.7

Assay was performed for marketed silodal tablets and the % purity was found to be 106.4%. (Table.5)

Table 5. Assay results of marketed tablet

Brand name	Name of the drug	Functional group	Absorbance	Label claim (mg)		% Purity
				Actual	Found	
Silodal (8mg)	Silodosin	$1637cm^{-1}$	1.05	300	294	106.4

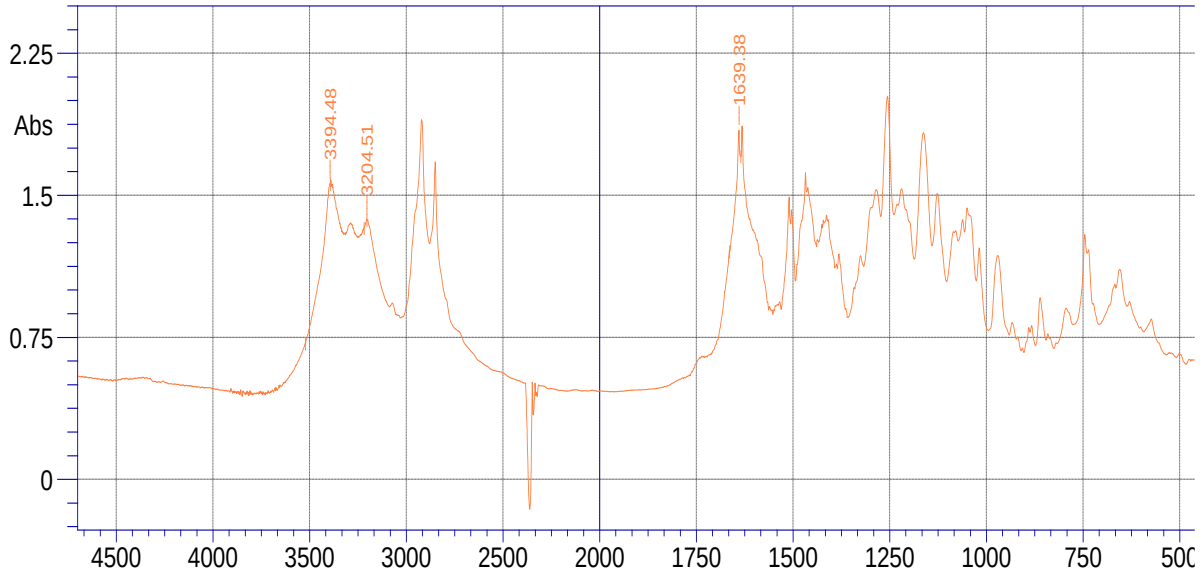


Figure 5. FTIR spectrum of assay of marketed formulation

Comparative studies of silodosin using HPLC

We then used ammonium acetate and acetonitrile (60:40) as the mobile phase in an RP-HPLC test for silodosin. The residue from the extracted tablet was diluted in 1000 ml acetonitrile and spiked in 10 ml acetonitrile to make standard stock solutions of 100 /ml each. The chromatogram was obtained after injecting these solutions into RP-HPLC.(Figure.6)

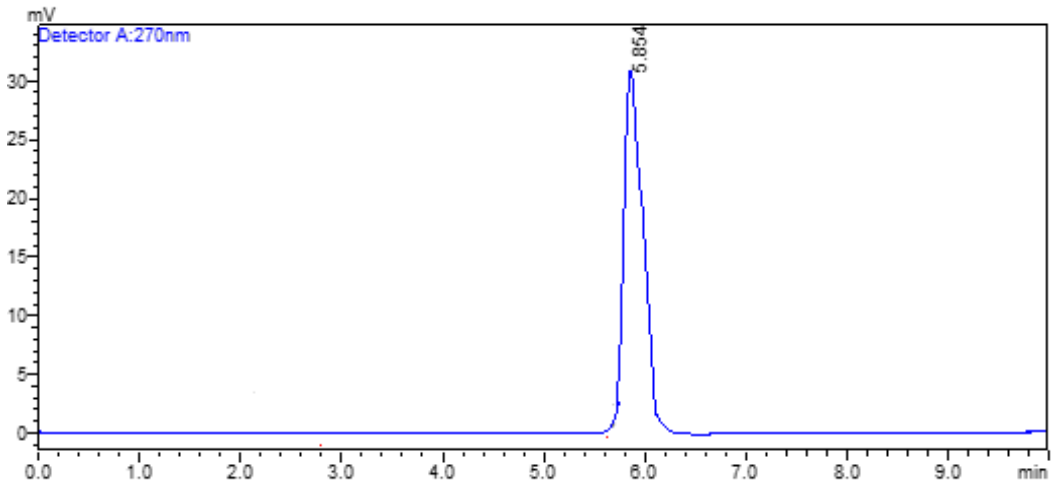


Figure 6: HPLC spectrum for assay of marketed formulation

Statistical analysis for FTIR v/s RP-HPLC

Student *t*-test

Assay results of silodosin were calculated by both methods. Statistical analysis of the results of two techniques showed significant difference between the methods at a significance level of (α) of 5% ($t_{calculated} > t_{critical}$). Furthermore, the amount of silodosin calculated by both methods was within the range between 90-110%.

Table 6. Statistical data for *t* –test of percentage purity of silodosin

Method	Mean of percentage purity	Standard deviation of individual data	Size of sample
FTIR	$\bar{x}_1$ =106.4	s_1^2=1.44	n_1 =3
RP-HPLC	$\bar{x}_2$ =101.7	s_2^2 =0.96	n_2 =3

Hypothesis: The two analytical methods to determine percentage purity of Silodosin are not significantly different.

$$H_0 : \mu = \mu_0$$

$$\text{Against } H_1 : \mu \neq \mu_0$$

Since variances of the population were not known and size of the samples was small, *t*-test for difference in means was adopted assuming the populations to be normal and the test statistic *t* were worked out under the given formula:

$$t = \frac{\overline{x_1} - \bar{x}_2}{\sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{(n_1+n_2-2)}} \times \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

P value (Probability of rejection) = 0.05 (two – tailed)

$t_{calculated}$= 5.66

$t_{critical}$ (0.05) = 2.776

Degrees of freedom (df) =n_1+ n_2−2 ; (3+3−2) = 4

As our hypothesis was two-sided, we applied two-tailed test for determining the rejection regions at 5 per cent level which came to as under, using table of *t*-distribution for 4 degrees of freedom:

R: | *t* | > 2.776

The observed value of R: | *t* | > 2.776 was 5.66 ($t_{calculated}$> $t_{critical}$) which falls in the region of rejection of our hypothesis. So, we reject our hypothesis of both methods not being significantly different and conclude that the two methods to determine percentage purity of Silodosin differ significantly.

Conclusion:

FTIR spectroscopy is utilized for qualitative analysis and is commonly accepted for the identification of functional groups in raw materials and final products, as referenced in all compendium pharmacopoeias. In quantitative determination, however, the development of a trustworthy FTIR approach with substantial derivative capabilities is beneficial. The proposed approach for silodosin estimation is based on the use of FTIR with a solid pellet methodology. The produced new technique was statistically compared to the pharmacopoeial method (HPLC), and the findings indicated that the developed new approach differed considerably. As a result, it has a wide range of applicability. It met all validation standards across a wide range of concentrations and can be used instead of approved procedures. It is appropriate for quality control of both pure and marketed solid dosage forms, and comparable techniques for estimating additional drug categories in formulations can be developed.

REFERENCES

1. *Montorsi F. [Profile of silodosin]. Urologiia. **(2013)** Mar-Apr;(2):112-4, 116-7. Russian. PMID: 23789376.*
2. *Tripathi KD. Essentials of medical pharmacology. JP Medical Ltd; **(2013)** Sep 30.*
3. *Shaik JV, Saladi S, Sait SS. Development of stability indicating UHPLC method for the quantitative determination of silodosin and its related substances. Journal of chromatographic science. **(2014)** Aug 1;52(7):646-53.*
4. *Er E, Erk N. An effective and sensitive stability-indicating chromatographic approach based on HPLC for silodosin assay. Journal of Analytical Science and Technology. **(2016)** Dec;7(1):1-8.*
5. *TP A, Rajasekaran A. Method development and validation for the estimation of sildosin in bulk and pharmaceutical dosage forms using UV-VIS spectrophotometry. Asian Journal of Pharmaceutical and Clinical Research. **(2012)**;5(4):150-2.*
6. *Goud VM, Rao AS, Ranjan SP, Shalini SD, Sowmya S, Bhoga B. Method development and validation of RP-HPLC method for assay of Sildosin in pharmaceutical dosage form. Inter. J. Pharma. Sci. **(2013)** Mar; 3:194-6.*
7. *Boltia SA, Abdelkawy M, Mohamed TA, Mostafa NN. Validated Chromatographic and Spectrofluorimetric Methods for Analysis of Silodosin: A Comparative Study with Application of RP-HPLC in the Kinetic Investigation of Silodosin Degradation. Journal of AOAC International. **(2020)** Jul 1;103(4):946-57.*
8. *Rao JV, Hotha KK, Somerset L. New rapid UPLC method for the estimation of impurities in the capsule dosage form of silodosin.*
9. *PrachiBhamre SJ. Spectrofluorimetric method for the determination of silodosin in bulk and pharmaceutical dosage form. Indo Am. J. Pharm. Res. **(2014)**; 4:5106-10.*
10. *Sayana PS, Iyer RS, Shibi A, Harischandran S. Development and validation of HPTLC method for quantification of silodosin in bulk and pharmaceutical dosage form. The Pharma Innovation. **(2012)** Dec 1;1(10).*
11. *Pandey S, Pandey P, Tiwari G, Tiwari R, Rai AK. FTIR spectroscopy: A tool for quantitative analysis of ciprofloxacin in tablets. Indian Journal of Pharmaceutical Sciences. **(2012)** Jan;74(1):86.*
12. *Kogawa AC, Salgado HR. Development and validation of infrared spectroscopy method for the determination of darunavir in tablets. Physical Chemistry. **(2013)**;3(1):1-6.*
13. *Nugrahani I, Musaddah M. Development and validation analysis of acyclovir tablet content determination method using FTIR. Int J Appl Pharm. **(2016)** May 3;8(Suppl 2):43-7.*
14. *Mallah MA, Sherazi ST, Bhanger MI, Mahesar SA, Bajeer MA. A rapid Fourier-transform infrared (FTIR) spectroscopic method for direct quantification of paracetamol content in solid pharmaceutical formulations. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. **(2015)** Apr 15; 141:64-70.*
15. *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. International Conference on Harmonization, Q2 (R1**):** Validation of Analytical Procedures: Text and Methodology. India. **(2005)**.*