

## A Green Approach for the Determination of Selected Anti-Diabetic Drugs in Pharmaceutical Formulation by Transmission FTIR Spectroscopy

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Métodos baseados em espectroscopia de infravermelho com transformada de Fourier (FTIR) para a determinação de duas drogas anti-diabetes, i.e., metformina HCl e glimepirida em formulações de comprimidos, são descritos. Os métodos envolvem o uso de discos de KBr, cuidadosamente preparados, com padrões e amostras de metformina HCl e glimepirida. Espectros de FTIR foram adquiridos na região de infravermelho média. A extração e pré-concentração foram evitadas. Para a quantificação da metformina HCl, uma banda de IR proeminente, com localização fixa em 3173 cm<sup>-1</sup> com dois pontos na linha base, entre 3550-2850 cm<sup>-1</sup> e para a glimepirida, uma banda com localização fixa em 1708 cm<sup>-1</sup>, com dois pontos na linha base, entre 1800-1600 cm<sup>-1</sup> foram selecionados para a calibração usando a lei de Beer. Para a metformina HCl, desenvolveu-se calibrações usando padrões na faixa entre 0,300 mg a 3,100 mg, enquanto que para a glimepirida, as calibrações foram desenvolvidas usando padrões na faixa entre 0,400 a 2,450 mg. Precisão e reprodutibilidade dos métodos propostos foram avaliadas e classificadas como satisfatórias com coeficientes de regressão excelentes, recuperação aceitável e baixos desvios do padrão. Os métodos de FTIR foram aplicados com sucesso na quantificação de formulações de fármacos contendo metformina HCl e glimepirida como substâncias ativas e os resultados comparáveis aos valores citados.

Fourier transform infrared spectroscopy (FTIR) based methods for the determination of two anti-diabetic drugs, i.e., metformin HCl and glimepiride in tablet formulation are described. The methods involve the use of carefully prepared KBr discs with standards and samples of metformin HCl and glimepiride. FTIR spectra were acquired in the mid IR region. Extraction and pre concentration procedures were totally avoided. For quantification of metformin HCl, a prominent IR band appearing at fixed location height 3173 cm<sup>-1</sup> with two point baseline between 3550-2850 cm<sup>-1</sup> and for glimepiride IR band at fixed location height 1708 cm<sup>-1</sup> with two point baseline between 1800-1600 cm<sup>-1</sup> were selected for Beer's law calibrations. For metformin HCl, calibrations were developed using standards in the range between 0.300 mg to 3.100 mg whereas for glimepiride, calibrations were developed using standards in the range between 0.400 mg to 2.450 mg. Accuracy and reproducibility of proposed methods were evaluated and found to be satisfactory with excellent regression coefficients, acceptable recoveries and low standard deviations. The FTIR methods were successfully applied for the quantification of pharmaceutical tablets formulation containing metformin HCl and glimepiride as active substances and the results were found to be comparable with the quoted values.

**Keywords:** transmission FTIR spectroscopy, metformin HCl, glimepiride, tablet formulations

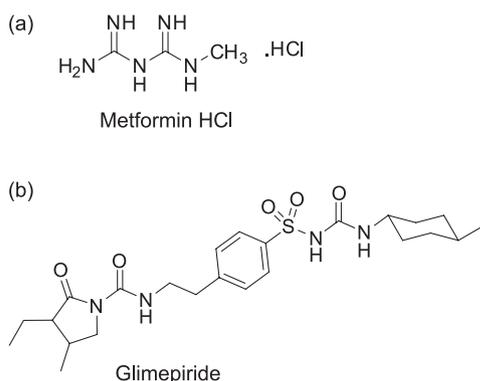
### Introduction

Type II diabetes is a metabolic disease, which is characterized by high blood sugar level. It is a life threatening disorder and prevalence of diabetes in developing countries are

penetrating very fast. One of the surveys reveals that 366 million cases will be predicted in 2030 and number of cases will be doubled from 2000 to 2030 in developing countries.<sup>1</sup> The initial development of type II diabetic condition is managed with the following key factors diet, life style modification, inherit, mental stress, and exercise. Oral therapy is initiated if the non-pharmacological means fail to control the disease.

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Metformin HCl (imidodicarbonimidicdiamide, *N,N*-dimethyl-monohydrochloride) is the first line of therapy in the case of overweight patients.<sup>2</sup> If blood glucose levels remains inadequate, so other oral hypoglycemic agents [sulfonyleureas usually glimepiride (3-ethyl-4-methyl-*N*-(4-[*N*-((1*r*,4*r*)-4-methylcyclohexylcarbamoyl)-sulfamoyl]phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide)] may be added in the therapy or review the dose of the metformin HCl<sup>2</sup> will be the strategy for controlling the levels of glucose in blood. Chemical structures of metformin HCl and glimepiride are presented in Figure 1a and 1b, respectively.



**Figure 1.** Structure of (a) metformin HCl and (b) glimepiride, respectively.

Different analytical methods, including chromatographic<sup>3-10</sup> and spectroscopic,<sup>11-20</sup> are reported for determination of metformin HCl and glimepiride in pharmaceutical dosage. Official methods for the determination of metformin HCl and glimepiride are available in British,<sup>21</sup> and US<sup>22</sup> Pharmacopeia. A review of the use of various analytical techniques for analysis of glimepiride has also been published in the past.<sup>23</sup> Most of these methods are lengthy, expensive, time consuming and laborious besides requiring a toxic solvents thus resulting in retardation in the pace of quantification. Moreover for betterment of human health, USP recommended that there should be a less use of chemicals and reagents in pharmaceutical analysis, so as all the above mentioned methods for detection of active will be less favorable for daily analysis of pharmaceutical dosage form.

Fourier transform infrared spectroscopy (FTIR), in recent years, has proved to be a simple, rapid and green method for the quantification of many active constituents in pharmaceutical samples,<sup>24-33</sup> as in most of the cases, no prior sample preparation is required. In the present work, metformin HCl and glimepiride, the two well-known anti-diabetic drugs, have been evaluated directly by transmission FTIR in dosage form by preparing accurately weighed amount of KBr pellets with standards and samples.

## Experimental

### Material and methods

#### Chemical and reagents

Pure analytical grade standard sample of metformin HCl and glimepiride were obtained with courtesy of local Pharmaceutical Suppliers. Spectroscopic grade KBr (Merck Company) was used for drawing pellet of standards and samples to analyse on the FTIR. Locally manufactured pharmaceutical formulations containing metformin HCl and glimepiride as an active ingredient are used for analysis. The method is outlined as follow:

#### Preparation of sample for analysis

Calibration and validation samples of metformin HCl and glimepiride were grinded in a mortar to homogenize the powders properly. Suitable weight ratios (such as 1 mg of metformin HCl and 0.500 mg of glimepiride with 99 mg of KBr) were transferred to 13 mm die and pressed near 12000 lbs for 5 min to obtain a pellet. The pellet was then placed in the FTIR for recording the spectra. The commercial tablets were first ground and then processed further in the same way as the calibration samples.

#### FTIR parameters

FTIR spectrometer of Thermo Nicolet 6700 with removable KBr optics and equipped with Deuterated Tri Glycine Sulfate (DTGS) detector was used to analyze pellets of standards and sample with KBr. All spectra were taken in mid infrared (IR) region of 4000-400  $\text{cm}^{-1}$  accumulating 32 scans *per* spectrum at an optimum resolution of 4  $\text{cm}^{-1}$ . Software named as OMNIC version 7.3 (Thermo Nicolet Analytical Instruments, Madison, WI) was used to record and operate FTIR spectra. Before each analysis, fresh background spectrum was taken with same instrumental conditions.

#### Calibrations

##### For metformin HCl

A set of eight standards with KBr in the form of pellet were prepared in concentration range of 0.300 mg to 3.100 mg for Beer's law calibration. Linear calibrations were obtained with chemometric software, Turbo Quant (TQ) Analyst.

##### For glimepiride

Similarly for glimepiride, a set of nine standards in the concentration range of 0.400 mg to 2.450 mg were prepared. Calibration was carried out by focusing on

carbonyl band at  $1708\text{ cm}^{-1}$  to achieve best linearity and regression results. Although other bands were also selected for the calibration but statistical results of selected bands were excellent.

#### Software and data treatment

Nicolet TQ Analyst chemometric software was used to construct Beer's law. To evaluate the predictive ability of the developed models and to compare it, the relative standard errors of prediction (RSEP) were calculated. The  $\text{RSEP}_{\text{CAL}}$  and  $\text{RSEP}_{\text{VAL}}$  errors were determined for the calibration and validation data sets, respectively, which are shown in Figure 3 and 5. To check the performance of the developed model the cross-validation technique, leave-one-out, was applied.

#### Interday and intraday precision

The intra and interday precision were checked assay of the samples on the same day and on different days at different time intervals, respectively.

#### Roughness of methods

The robustness of the method was evaluated by assay of the sample, by a different analyst on same instrument on a different day.

#### Efficiency and recovery

Through standard addition method, the recovery was determined to check the efficiency of method. In this method, varying quantities of the standards were added to the known concentration of pharmaceutical sample and then by the proposed method total concentration was evaluated. Then this concentration is compared with the actual concentration. The recovery and efficiency was calculated by using equation 1.

$$R (\%) = (C.B/A) \times 100 \quad (1)$$

where R is concentration of glimepiride recovered, C is total concentration after addition, B is the concentration of sample taken before addition and A is concentration of standard added.

#### Limit of detection and quantification

The analytical parameters, limit of detection (LOD) and quantification (LOQ) for the current method were

determined by calculating the peak height of the IR bands at  $3173\text{ cm}^{-1}$  for metformin HCl and at  $1708\text{ cm}^{-1}$  in glimepiride at a fairly low concentration until the band just disappeared. The lowest amount from which the substantial signal was produced was analyzed eleven times and calculated using the equation 2. Likewise, LOQ of this method was determined with the equation 3.

$$\text{LOD} = 3 \times \text{SD} \times C/P \quad (2)$$

where SD is standard deviation, C is the lowest concentration of standards that could be detected and P is the peak height.

Similarly:

$$\text{LOQ} = 10 \times \text{SD} \times C/P \quad (3)$$

In this study, AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals,<sup>34</sup> were used to calculate the coefficient of variation (CV, %) of the data set and used as relative standard deviation (RSD, %).

## Results and Discussion

Metformin HCl and glimepiride have been successfully determined in this study by transmission FTIR using KBr pellets. Proposed methods for assessment of metformin HCl and glimepiride will be a worthwhile addition to the quality control and quality assurance in pharmaceutical industry.

FTIR spectra of pure standard and a drug sample containing metformin hydrochloride are shown in Figure 2a and 2b, respectively. Two characteristic bands of metformin hydrochloride were observed at  $3370\text{ cm}^{-1}$  and  $3292\text{ cm}^{-1}$  relative to the N-H primary stretching vibration. While a band at  $3173\text{ cm}^{-1}$  due to the N-H secondary stretching, and characteristic bands at  $1622\text{ cm}^{-1}$  and  $1568\text{ cm}^{-1}$  are assigned to C-N stretching. As the amount of metformin hydrochloride in the tablet was above 95%, there is no major interference of the matrix substances. Both spectra related to standard and drug sample of metformin hydrochloride were found to be comparable which is very obvious from Figure 2a and 2b.

Many bands and spectral regions were selected for the calibrations. However, selected bands and spectral regions provided best results for Beer's law calibrations. KBr disks with various concentrations of the metformin HCl standards in ranging between 0.300 mg to 3.100 mg were prepared. A band showing best results at  $3173\text{ cm}^{-1}$  for Beer's law with  $R^2$  value of 0.996 and spectral region between  $3550\text{ cm}^{-1}$  to  $2850\text{ cm}^{-1}$  (the absorbance of N-H primary and secondary stretching vibrations).

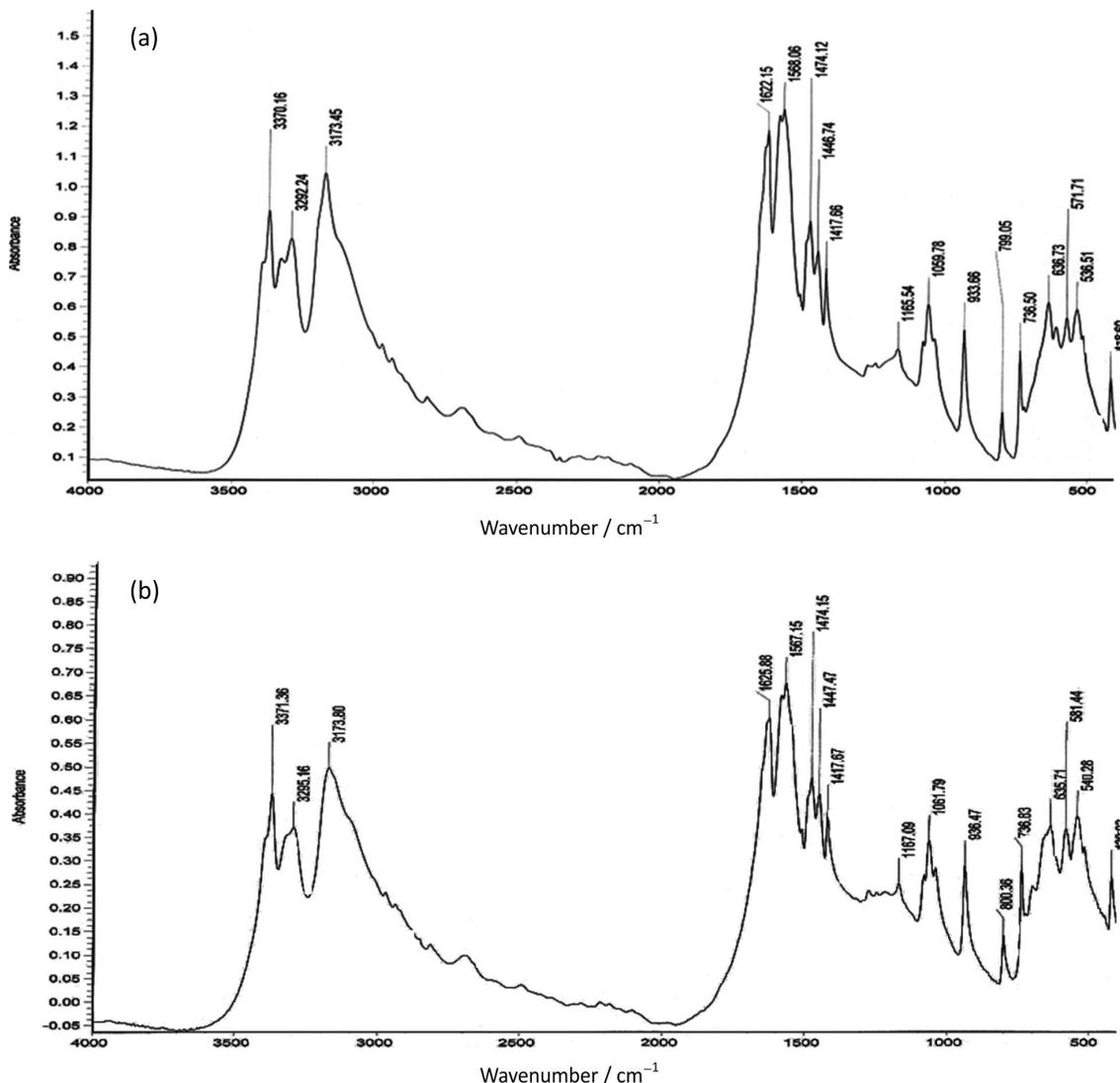


Figure 2. FTIR spectra of (a) pure standard metformin HCl and (b) as drug sample (glucophage).

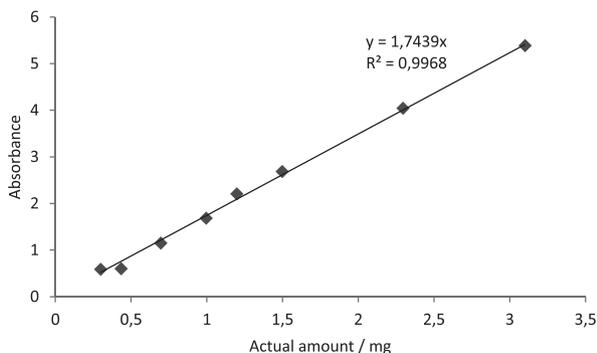


Figure 3. Beer's law calibration of metformin HCl.

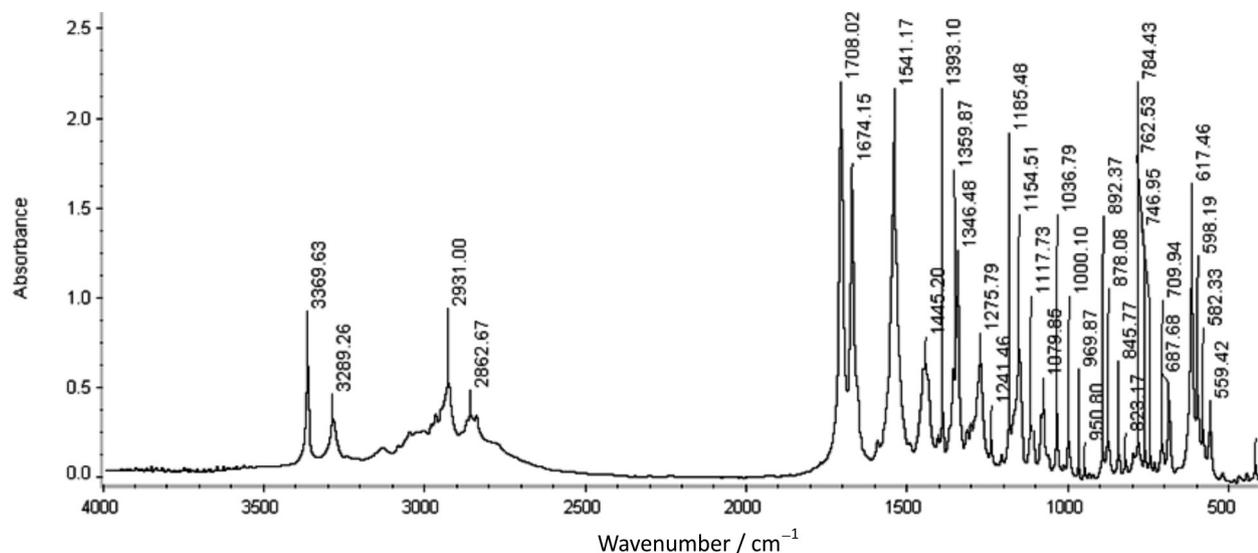
The Beer's law calibration model was developed through computed fixed height in the TQ analyst program. Following achieved regression equation (equation 4) was applied on tablets obtained from the local commercial drug stores to calculate actual amount of metformin HCl.

$$Y = 1.7439 x + 0.000 \tag{4}$$

The quantitative results of metformin HCl were also checked by selecting another relatively sharp band at 936.46 cm<sup>-1</sup>, which is very clear in Figure 2a and 2b, but calibration was not so linear as compared to calibration of 3173 cm<sup>-1</sup> band.

An additional advantage of the comparison of Figure 2a and 2b is that it provides an opportunity to check the presence of impurity in drug samples as several substandard or expired drugs may be present in the market; hence it is well suited to distinguish even counterfeit drugs from the original sample. This quality is specific for a direct FTIR method, such as present one, over the generally used spectrophotometric and chromatographic methods.

Characteristic stretching bands at 3369 cm<sup>-1</sup> correspond to amine (-N-H), a band at 2931 cm<sup>-1</sup> relates to aromatic

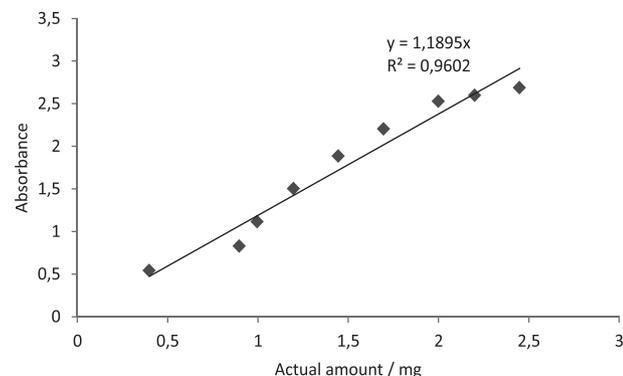


**Figure 4.** Representative FTIR spectrum of drug sample of glimepiride.

(-C-H) while bands at 1708, 1079 and 1544  $\text{cm}^{-1}$  correspond to amide (C=O), sulfoxides and aromatic (C=C), respectively.

From various selected bands, a specific band at 1708  $\text{cm}^{-1}$  with baseline from 1800 to 1600 due to carbonyl group of glimepiride was selected for Beer's law calibration because bands due to amine groups are not so intensive. Therefore, it has not provided good calibration for our selected range of standards. The concentration range of glimepiride standards for Beer's law was ranged between 0.400 mg to 2.450 mg in KBr pellets with the regression coefficient  $R^2 = 0.960$ . The result of calibration was found to be acceptable. As concentration of standards was increased, absorbance was found to increase accordingly. So, without any doubt, such calibrations could be boldly used for accurate quantification of active component in pharmaceutical dosage. The Beer's law calibration model is shown in Figure 5.

Following regression equation (equation 5) was achieved through Beer's law calibration plot, which was



**Figure 5.** Beer's Law calibration of glimepiride.

applied on the real pharmaceutical samples to calculate actual amount of glimepiride in tablet formulation.

$$Y = 1.1895 x + 0.000 \quad (5)$$

The LOD and LOQ for metformin HCl were determined and found to be 0.138 mg and 0.572 mg, respectively. Similarly, for glimepiride these values were calculated as 0.001 and 0.006 respectively.

Beer's law calibrations were applied on real pharmaceutical samples containing metformin HCl and glimepiride as active ingredients. FTIR predictions given in Table 1 indicated that amount of active ingredient is almost comparable with quoted values on the packet of tablets.

**Table 1.** FTIR quantification of metformin HCl and glimepiride in pharmaceutical samples by the proposed method

Metformin HCl		
Product No.	Amount labeled <sup>a</sup> / mg	Amount found <sup>b</sup> / mg
Sample 1	500.0	493.5
Sample 2	500.0	504.0
Sample 3	850.0	793.9
Glimepiride		
Sample 1	4.000	4.208
Sample 2	4.000	4.000
Sample 3	4.000	4.004

<sup>a</sup>Amount of Metformin HCl per tablet labeled on package; <sup>b</sup>mean value of samples (n = 3) and SD ( $\pm$ ) was < 5%.

The FTIR methods were further evaluated by standard addition method and results obtained from the study are summarized in Table 2. These were calculated in triplicate

**Table 2.** Recovery test of metformin HCl and glimepiride by standard addition method

Metformin HCl						
Sample No.	Amount of drug in sample / mg	Amount of standard added / mg	Total amount / mg	Amount found / mg	Recovery $\pm$ S.D. / %	Acceptable recovery / % <sup>34</sup>
1	2.390	0.991	3.380	3.290	97.40 $\pm$ 0.230	
2	1.910	0.990	2.900	2.896	99.88 $\pm$ 0.496	90-108
3	1.370	1.370	2.750	2.700	98.29 $\pm$ 0.230	
Glimepiride						
1	1.450	1.000	2.450	2.500	102.0 $\pm$ 0.127	
2	1.590	0.951	2.540	2.480	97.83 $\pm$ 0.423	90-108
3	1.660	1.000	2.660	2.600	98.04 $\pm$ 0.309	

**Table 3.** Precision data of the method

Metformin HCl					
Actual amount 3.380mg		Actual amount 2.900mg		Actual amount 2.750 mg	
Observed / mg	Recovery $\pm$ S.D. / %	Observed / mg	Recovery $\pm$ S.D. / %	Observed / mg	Recovery $\pm$ S.D. / %
Intraday					
3.290	97.40 $\pm$ 0.230	2.896	99.88 $\pm$ 0.496	2.600	98.04 $\pm$ 0.309
Interday					
3.310	97.92 $\pm$ 0.367	2.910	100.3 $\pm$ 0.386	2.700	98.18 $\pm$ 0.476
Glimepiride					
Actual amount 2.450mg		Actual amount 2.540mg		Actual amount 2.660	
Intraday					
2.500	102.0 $\pm$ 0.127	2.480	97.83 $\pm$ 0.423	2.600	98.04 $\pm$ 0.309
Interday					
2.400	97.95 $\pm$ 0.347	2.500	98.42 $\pm$ 0.489	2.675	100.5 $\pm$ 0.532

**Table 4.** Data of ruggedness studies

Metformin HCl					
Actual amount 3.380mg		Actual amount 2.900mg		Actual amount 2.750 mg	
Observed / mg	Recovery $\pm$ S.D. / %	Observed / mg	Recovery $\pm$ S.D. / %	Observed / mg	Recovery $\pm$ S.D. / %
Analyst 1					
3.290	97.40 $\pm$ 0.230	2.896	99.88 $\pm$ 0.496	2.600	98.04 $\pm$ 0.309
Analyst 2					
3.310	97.92 $\pm$ 0.367	2.910	100.3 $\pm$ 0.386	2.700	98.18 $\pm$ 0.476
Glimepiride					
Actual amount 2.450mg		Actual amount 2.540mg		Actual amount 2.660	
Analyst 1					
2.500	102.0 $\pm$ 0.127	2.480	97.83 $\pm$ 0.423	2.600	98.04 $\pm$ 0.309
Analyst 2					
2.400	97.95 $\pm$ 0.347	2.500	98.42 $\pm$ 0.489	2.675	100.5 $\pm$ 0.532

and mean values are quoted with standard deviation (SD). These results revealed excellent precision and accuracy of developed methods.

Results of intermediate precision and ruggedness are shown in Table 3 and 4, respectively. The standard deviation and recoveries for the both methods were found

to be excellent indicating high degree of precision of the methods. The % recovery was between 97-102% indicating high degree of accuracy.

## Conclusions

The present study reported simple, rapid and green methods for the analysis of two antidiabetic drugs, i.e., metformin HCl and glimepiride. Proposed methods are suitable for QC process in pharma industry. Therefore, as compared to other spectroscopic or chromatographic method, this is a direct, inexpensive and green approach in which costly chemicals and toxic solvents are totally avoided. Such types of FTIR applications have a strong potential to replace classical methods in quality assurance /quality control (QA/QC) for the analysis of active contents in pharmaceutical preparations.

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