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In-Depth qualitative and quantitative FTIR spectroscopic study of glipizide and gliclazide

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ABSTRACT

The structural information of the active pharmaceutical ingredients and in-depth insights on the modes of molecular interactions is very important aspects in drug development. In the present work, the process of the molecular self-assembly of two sulfonylurea oral hypoglycemic drugs, glipizide and gliclazide, has been studied in the solid-state using FTIR spectroscopy. Interaction patterns in the respective crystalline states were obtained from the single crystal data. Quantitation of the studied drugs and their binary mixtures has been performed by integrating the peak areas of the characteristic well resolved CCC bending and ring deformation bands at 1527 and 706 cm^{-1} for glipizide and gliclazide, respectively. The results from this study provide data that can be used for the preparative process monitoring of glipizide and gliclazide in various dosage forms, and their interactions with pharmaceutical excipients.

KEYWORDS

Glipizide;
Gliclazide;
FTIR;
Pharmaceuticals;
Sulfonylurea.

INTRODUCTION

Glipizide (N-[2-[4-[[[(cyclohexylamino) carbonyl] amino]sulfonyl]phenyl]ethyl] -5- methyl pyrazinecarboxamide); GPZ, (Figure 1a) and gliclazide (N-(hexahydrocyclopenta[c]pyrrol-2(1H)-ylcarbamoyl)-4-methyl benzene sulfonamide); GCZ, (Figure 1b) are oral medium-to-long acting sulfonylurea hypoglycemic drugs. They are acting by partially blocking potassium channels in the beta cells of the islets of Langerhans. Thus, they will increase the time that the cell spends in the calcium release stage of cell signaling leading to an increase in calcium, which will initiate more insulin release from each beta cell[1].

Because of their wide use, several methods have been reported for the determination of GPZ and GCZ in bulk, pharmaceutical dosage forms and/or biological

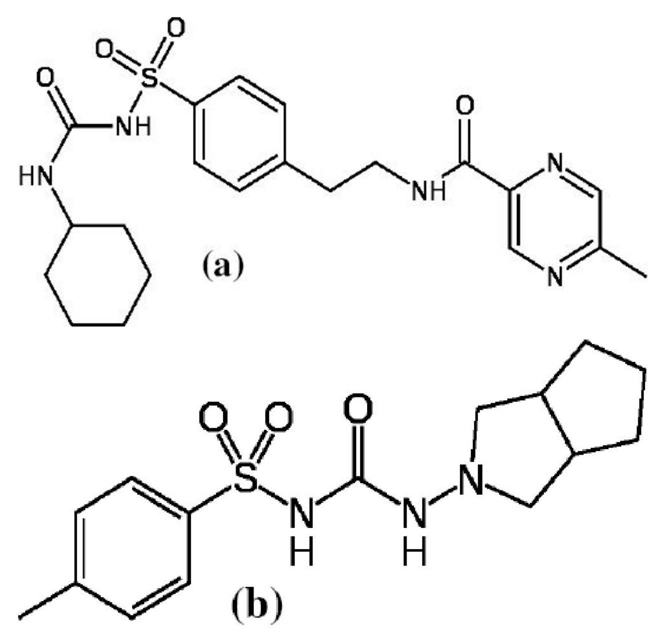

Figure 1 : The chemical structures of GPZ (a) and GCZ (b).

fluids. These methods include but not limited to titrimetry[2], spectrophotometry[3-9], fluorimetry[3,10], electrochemical methods[11-13], TLC[14,15], HPLC[16-23], capillary electrophoreisis[24-27], and immunoassay[28,29].

Infrared spectroscopy is a vibrational spectroscopic technique classified within the Category I of analytical methods according to the United States Pharmacopeia, USP,[30]. FTIR spectroscopic technique has been rapidly revolutionized and widely applied in various disciplines that include the chemical, physical, pharmaceutical, medical and natural sciences. The unique chemical fingerprinting capability of this technique has led to extensive opportunity for fundamental applications in the pharmaceutical arena. It plays a significant role in providing specific information on the identification and characterization of materials, as well as providing fundamental molecular spectroscopic information in the structural elucidation of unknown compounds. It has been successfully applied for quantitation of pharmaceuticals[31-37]. Recently, FTIR spectroscopy has been included in British Pharmacopeia, (BP)[2] to identify both GPZ and GCZ.

The aim of the present work is to utilize FTIR spectroscopy to investigate the molecular self-assembly of both GPZ and GCZ in the light of their available single crystal data[38-40] and quantify the studied drugs and their binary mixtures.

EXPERIMENTAL

Chemicals

Glipizide and gliclazide were purchased from Sigma Aldrich Gillingham Dorset, England. Potassium bromide (El-Nasr Pharmaceutical Chemical Co., Abo-Zaabal, Egypt). Chloroform (Merck KGaA, Darmstadt, Germany). Other chemicals were of analytical grade and used as received.

Pharmaceuticals

Minidiab® tablets (Chemical Industrial Development for Farmitalia Carlo Erba, Cairo, Egypt) labeled to contain 5 mg of GPZ per tablet. Diamicron® tablets (Servier Egypt Industries, Cairo, Egypt) labeled to contain 80 mg of GCZ per tablet.

Preparation of standard solutions

The stock standard solutions (5 mg ml^{-1}) were prepared by dissolving an accurately weighed amount (250 mg) of each drug in 50 ml chloroform[35]. The working standard solutions were obtained by further dilution of these stock solutions with the same solvent.

Treatment of the pharmaceutical dosage forms

The extraction of the investigated drugs from their tablets was achieved through the selective dissolution of GPZ and GCZ with chloroform[2]. Ten tablets were weighed, and finely powdered. An accurately weighed quantity of the powdered tablet equivalent to 250 mg of the drug was transferred into a 50 ml calibrated flask, and dissolved in about 25 ml of chloroform. The contents of the flask were swirled, sonicated for 5 min and then completed to the volume with chloroform. The mixtures were mixed and filtered. Different volumes of the prepared solution were diluted with chloroform, and the resulting solutions were used for analysis by the recommended procedures.

Preparation of the binary mixtures

Glipizide and gliclazide were physically mixed in various ratios. The calibration curves were constructed by plotting the average peak areas of the characteristic FTIR band as a function of the weight percentage (% w/w) in the range of (5 ~ 95 % w/w). The samples were analyzed in triplicate to determine the linearity of the constructed calibration curve.

General recommended procedures

One milliliter, accurately measured, of the standard or sample solution (2–100 mgml^{-1}) was poured into a 1000 mg KBr powder in porcelain dishes[31]. The dishes were kept in hot air oven for 25 min at 60 ºC to evaporate the moisture and solvent from the mixture[31]. The powders were thoroughly mixed with an agate pestle. Likewise, seven homogeneous samples were prepared and employed for quantitative measurement. Calibration graphs were constructed by plotting the obtained peak area of the characteristic FTIR band versus the corresponding drug concentrations.

Instrumentation

Infrared spectroscopy

FT-infrared spectra were collected in triplicate using a Nicolet 6700 FTIR Advanced Gold Spectrometer with OMNIC 8 software (Thermo Electron Scientific Instruments Corp., Madison, WI USA) and Jasco 6000 FTIR (Hachioji, Tokyo, Japan). The FTIR spectra of the investigated drugs were recorded as potassium bromide discs (1:200) in the range of 4000-400 cm^{-1} at 4 cm^{-1} spectral resolution with the accumulation of 256 spectral.

Single crystal data analysis

Single crystal data for the crystalline forms of GPZ and GCZ[38-40] with codes of SAXFED and SUVGUL, respectively, were obtained from the Cambridge Structural Database (CSD) using the ConQuest 1.13 software. The Mercury 3.0 program was used for the structural analysis (molecular packing) of these compounds. The information from the crystal structure were used to facilitate the interpretation of the FTIR bands.

Spectral preprocessing

All the FTIR spectra were exported to the Galactic SPC format and manipulated using GRAMS AI (Galactic Industries, Salem, NH, USA, version 7.01).

Spectrophotometry

Absorbances of the selected drugs were measured using UV-1601 PC (Shimadzu, Kyoto, Japan) and Lambda-3 B (Perkin-Elmer Corporation, Norwalk, USA) ultraviolet–visible spectrophotometers with matched 1 cm quartz cells were used for all measurements.

RESULTS AND DISCUSSION

FTIR spectra of GPZ and GCZ were recorded in the range of 4000-400 cm^{-1} using the transmittance mode of operation. The FTIR spectra of GPZ and GCZ are shown in (Figure 2). These spectra demonstrate clearly the complexity of the structural information that is obtained from the FTIR bands. The distinctive FTIR wavenumbers are listed in (TABLE 1). Assignment of the prominent FTIR features was carried out in the light of their available single crystal data[38-40], and the FTIR spectroscopic data previously reported[41,42].

Infrared spectroscopic investigations of GPZ and GCZ

The crystal structures of both GPZ and GCZ (Figure 3) have been reported[38-40]. The pattern of the hydrogen bonding is similar to that observed in a number of sulphonylureas, for which two main patterns are observed, one in which the sulphonylurea fragments are arranged in a parallel manner, and another in which the fragments are antiparallel. GCZ shows slightly stronger intermolecular hydrogen bonds than that observed in GPZ.

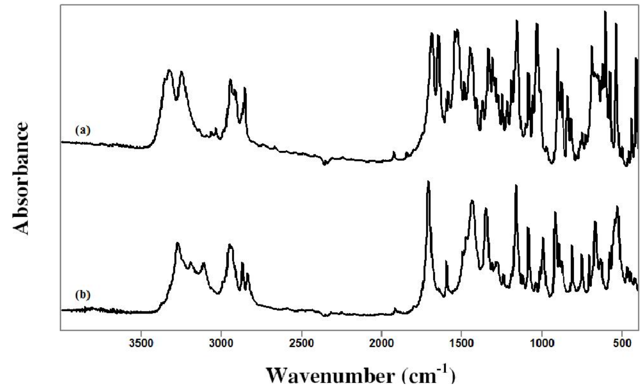

Figure 2 : The FTIR spectra of GPZ (a) and GCZ (b) in the region of 400-4000 cm^{-1}

Glipizide and GCZ can be readily differentiated by their FTIR spectroscopic signatures. The key FTIR spectral features of GPZ are υ $(NH)_{amide}$ band at 3326 cm^{-1}, υ $(NH)_{urea}$ band at 3249 cm^{-1}, υ (C=O) band at 1690 cm^{-1}, δ (CCC) band at 1527 cm^{-1}, δ (CCH) band at 1485 cm^{-1}, and δ (CO) band at 607 cm^{-1}. GCZ; in turn, is characterized by υ $(NH)_{urea}$ band at 3272 cm^{-1}, υ (C=O) band at 1710 cm^{-1}, υ (C-N) band at 1353 cm^{-1}, the out-of-plane δ (C-H) band at 997 cm^{-1}, and the ring torsion band at 706 cm^{-1}.

Quantitative determination and validation

The FTIR spectroscopy has been utilized for quantitative determination of GPZ and GCZ in their pure forms, tablets and binary mixtures. The δ (CCC) band at 1527 cm^{-1} and the ring torsion band at 706 cm^{-1}, respectively, were picked up for their quantitative determination because they are well resolved and free from interferences (Figure 4). The peak areas of the bands of interest were integrated using GRAMS AI (Galactic Industries, Salem, NH, USA, version 7.01) and the Gaussian and Lorentzian function. The developed procedures were validated according to USP *XXV* validation guidelines[30] and International Conference on Harmonization (ICH) guidelines[43]. The following validation parameters were studied:

Linearity, detection and quantitation limits

a) For GPZ and GCZ in their pure forms

The calibration graphs for GPZ and GCZ in their

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TABLE 1 : The distinctive FTIR wavenumbers (cm^{-1}) of GPZ and GCZ.

FTIR		Proposed assignment
GPZ	GCZ	
3354 *s*	3370 *w*	
3326 *s*		υ(NH)
3249 *s*	3272 *s*	υ(NH)
	3248 *m, sh*	υ(CH)
	3192 *m*	υ(CH)
3095 *mw*	3112 *ms*	υ(CH)
2948 *s*	2949 *s*	υ(CH) and C-C$_{ring}$ modes
2914 *s*	2929 *s*	υ(CH3) and C-C ring modes
2867 *m, sh*	2867 *ms*	combination: (C=O) in-plane and C-C$_{ring}$ modes
2855 s	2837 *m*	υ(CH)
1690 *vs*	1710 *vs*	υ(C=O)
1650 *vs*	1650 *vw*	υ(C=O)$_{amid}$
1616 *w*		υ(C=C)
1584 *ms*	1595 *ms*	υ(C-C)$_{ring}$
1540 *vs*		δ(CCC)
1527 *vs*		δ(CCC)
1485 *s*		δ(CCH)
1445 *vs*	1434 *vs*	δ(CH)
1376 *ms*		δ(CH_2)
1334 *s*	1353 *vs*	υ(C-N)
1248 *ms*	1240 *m*	in-plane δ(CH)
1158 *vs*	1163 *vs*	combination: υ(SO_2) and in-plane δ(CH)
1061 *m*		in-plane δ(CH)
1035 *vs*	1040 *mw*	in-plane δ(CH)
973 *mw*	997 *s*	out-of-plane δ(C-H)
903 *s*	919 *vs*	out-of-plane δ(C-H)
840 *m*		out –of-plane δ(C-H)$_{ring}$
817 *m*	811 *ms*	out-of-plane δ(CH)
727 *mw*	706 *m*	ring deformation
620 *mw*		in-plane ring deformation mode
607 *vs*		δ(CO)
	547 *ms*	in-plane δ(CCN)
539 *vs*	530 *vs*	in-plane δ(CC)
501 *mw*		in-plane δ(CC)$_{chain}$
	470 *m*	ring torsion
412 *s*	420 *mw*	out-of-plane δ(CCC)

***m, s, sh.* and *v* stand for medium, strong, shoulder and very. *υ* and *δ* stand for stretching and bending, respectively**

pure forms were constructed by analyzing a series of concentrations of the standard solutions of the drugs and then plotting the peak area as a function of their corresponding concentrations. In all cases, linear relationships were found between the measured values of peak areas and the concentrations of the investigated drugs. High correlation coefficients obtained (0.9987-0.9996) in the concentration range of 12-80 μg/mg. LODs were 4.00 and 11.8 μg/mg, while LOQs were

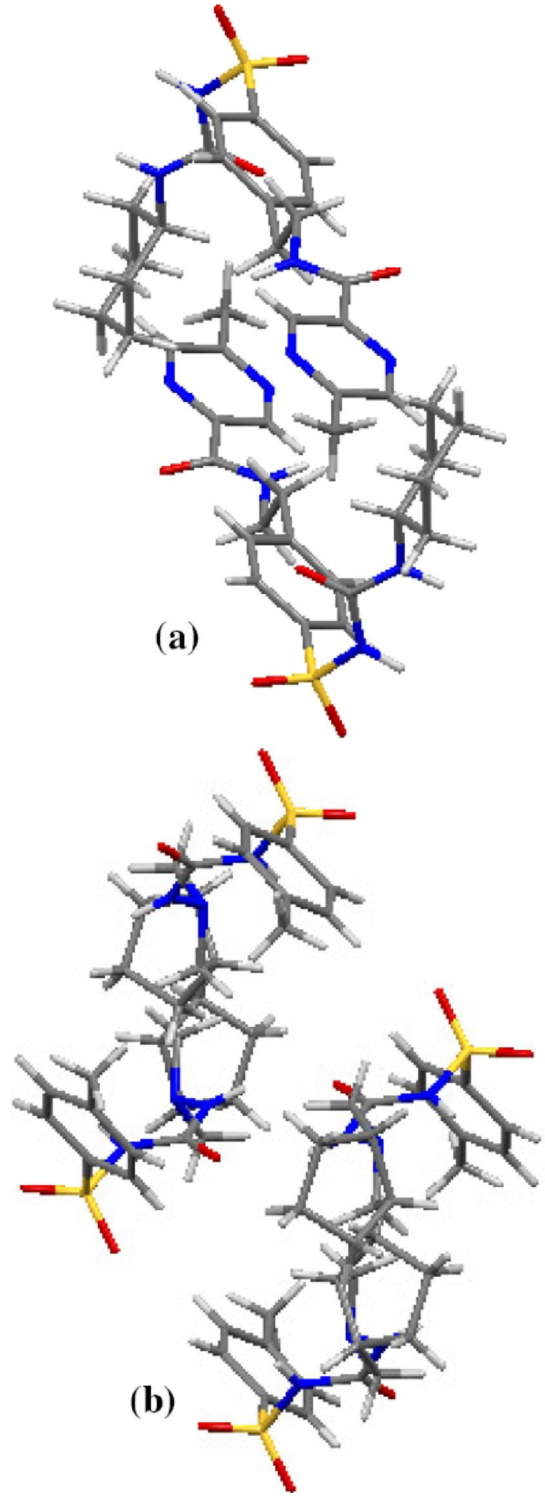

Figure 3 : Crystal structures of GPZ (a) and GCZ (b) [38-40]

12.0 and 35.5 μg/mg for GPZ and GCZ, respectively, (TABLE 2).

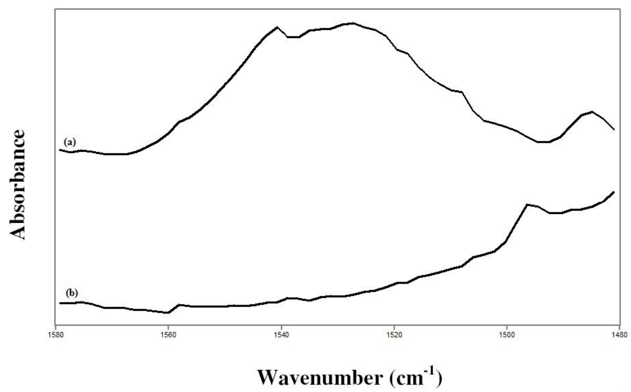

Figure 4a : The FTIR spectra of GPZ (a) and GCZ (b) in the region of 1480-1580 cm^{-1}

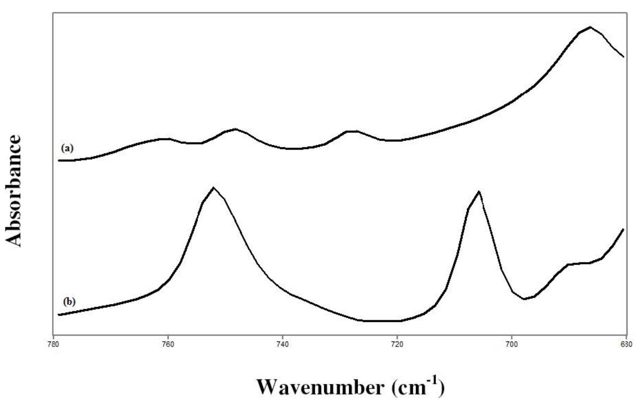

Figure 4b : The FTIR spectra of GPZ (a) and GCZ (b) in the region of 680-780 cm^{-1}.

b) For the binary mixtures

Similarly the calibration graphs for binary mixture of GPZ and GCZ were constructed by analyzing series of concentrations in the range of (5 ~ 95 % w/w). Linear relationships were found between the measured values of peak areas and the percentage of the investigated drugs as indicated by the high correlation coefficients obtained (0.9878-0.9968). LODs were 2.0 and 2.9 (% w/w), while LOQs were 6.0 and 8.8 (% w/w) for GPZ and GCZ, respectively, (TABLE 2).

Accuracy and precision

The accuracy of the proposed method was evaluated by the standard addition method. The recovery values of the added concentrations were 98.7–99.5±1.05–1.21% (TABLE 3). This indicated the accuracy of the method. The precision of the proposed method was also determined by performing replicate analysis of five samples of each drug. The coefficient of variation of root square mean deviation (CV (RSMD)) had not exceeded 2.5% indicating good reproducibility of the proposed methods (TABLE 4). This precision level is adequate for the routine analysis of the investigated drugs in quality control laboratories.

Robustness and ruggedness

Robustness was studied by evaluating the influence of small variations in the experimental parameters; time and drying temperature on the method performance. It was found that small variation in these variables had no significant effect on the method significantly (TABLE 5) which indicates the reliability of the proposed method during its routine application for analysis of the investigated drugs.

TABLE 2 : Quantitative parameters for the assay of the studied drugs by FTIR Spectroscopy.

Parameter [a]	GPZ (alone)	GCZ (alone)	GPZ (binary)	GCZ (binary)
Linear range	12-30 (μg/mg)	35-80 (μg/mg)	5 - 95 (% w/w)	5 - 95 (% w/w)
Intercept (a) ± RSMD	-0.03006 ± 0.0408	-0.01442 ± 0.0281	-0.2258 ± 0.1346	0.1366 ± 0.0535
Slope (b) ± RSMD	0.03379 ± 0.0027	0.00783 ± 0.00049	0.0657 ± 0.00235	0.0180 ± 0.0011
Correlation coefficient (r)	0.9987	0.9903	0.9968	0.9929
LOD	4.00 (μg/mg)	11.8 (μg/mg)	2.0 (% w/w)	2.9 (% w/w)
LOQ	12.0 (μg/mg)	35.5 (μg/mg)	6.0 (% w/w)	8.8 (% w/w)

[a] **n= three determinations LOD : limit of detection LOQ: limit of quantitation**

Ruggedness was also investigated by applying the proposed method to the assay of the investigated drugs using the same procedure but using two different instruments at two different laboratories, different elapsed time and different sample addition. The results obtained from lab- to-lab, day-to-day and different sample addition variations were found to be reproducible (TABLE 6).

Extraction and interference studies

Prior to the application of the proposed method on

TABLE 3 : Recovery of standard drugs added to their dosage forms using the proposed FTIR method

Drug	Dosage form	Declared amount (mg)	Added amount (mg)	Recovery (%±RSMD)^a
GPZ	Mindiab ®tablets	10	10	99.5 ± 1.21
GCZ	Diamicron® tablets	30	30	98.7 ± 1.05

^{a} **Values are the mean of three determinations**

the analysis of GPZ and GCZ in their tablets, The FTIR spectra of the tablets before and after (excipients only) the extraction of the drugs by chloroform were recorded (Figures. 5, 6). By close visual inspection of Figure 5, it can be noted that, the υ (C=O) band at 1690 cm^{-1} and the δ (CCC) band at 1527 cm^{-1} in case of GPZ (indicated by arrows in Figure 5) and the υ (CH) band at 2837 cm^{-1}, the υ (C=O) band at 1710 cm^{-1} and the υ $(C\text{-}C)_{ring}$ band at 1595 cm^{-1} in case of GCZ, (indicated by arrows in Figure 6) were recorded in the FTIR spectra of the tablets. The wavenumbers of these FTIR spectral bands were not recorded for the inactive pharmaceutical excipients contained in the tablets (Figures. 5, 6). Despite the presence of some FTIR spectral features arising from the inactive ingredients in the intact tablets, it is possible to directly identify and quantify GPZ and GCZ by their characteristic FTIR bands in these complex matrices.

TABLE 4 : Precision of the proposed FTIR method

Drug	Conc. (μg/mg)	Absorbance^a					Mean	RSMD^b	CV(RSMD^c (%)
		Sample number							
		1	2	3	4	5			
GPZ	20	0.597	0.630	0.627	0.636	0.612	0.620	0.0140	2.25
GCZ	60	0.400	0.393	0.384	0.376	0.399	0.390	0.0090	2.30

^{a}**Results are compared with those of standard calibration curves.;** ^{b}**RSMD: root square mean deviation.;** ^{c}**CV(RSMD): coefficient of variation of root square mean deviation**

TABLE 5 : Robustness of the proposed FTIR method

Parameter	Recovery (% ± RSMD) ^{a}	
	GPZ	GCZ
Optimum condition^b	100.1± 0.92	99.5 ± 0.68
Drying time (min)		
20	98.5 ± 0.75	100.5± 1.22
30	100.2 ± 1.12	98.5 ± 0.88
Drying temperature (°C)		
50	97.9 ± 0.67	99.3 ± 0.96
70	99.6 ± 0.75	98.5 ± 0.68

^{a} **Each value is the mean of three determinations.;** ^{b}**The condition were 1 ml of drug, drying time of 30 min at 60 °C.**

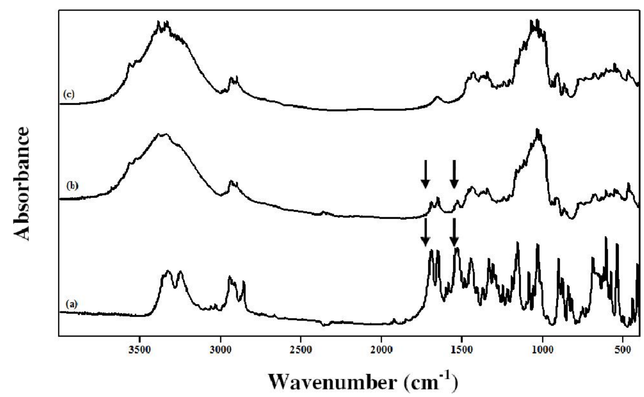

Figure 5 : The FTIR spectra of (a) pure GPZ, (b) tablet before and (c) after (excipients only) extraction in the region of 400-4000 cm^{-1}.

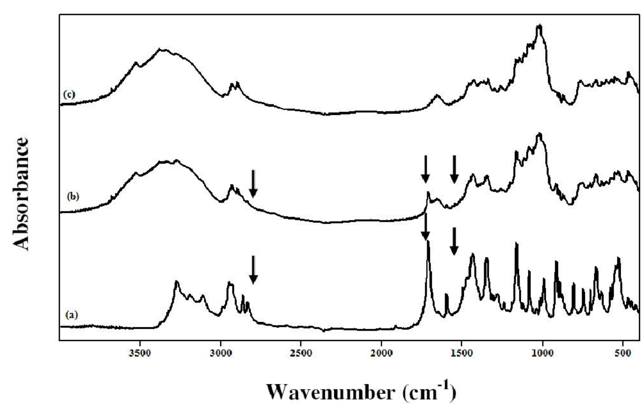

Figure 6 : The FTIR spectra of (a) pure GCZ, (b) tablet before and (c) after (excipients only) extraction in the region of 400-4000 cm^{-1}.

Application to the analysis of the pharmaceutical dosage forms

The proposed method was applied to the determination of GPZ and GCZ in their available tablets in the local market. The recovery percentages ranged from 98.1-100.6 0.85-1.20%. The results were compared with those obtained by the reported methods[4,7] (TABLE 7). No significant differences were found between the calculated and theoretical values of both the

TABLE 6 : Ruggedness of the proposed FTIR method

Drug	Recovery (% ± RSMD)[a]					
	Instrument		Inter-day variation		Sample addition	
	Nicolet 6700 FTIR	Jasco 6000 FTIR	1 day	2 day	Solution	Weighing
GPZ	99.3 ± 0.97	99.5 ± 1.11	99.3 ± 0.97	99.7 ± 0.55	99.3 ± 0.97	97.6 ± 0.69
GCZ	99.3 ± 0.77	99.8 ± 0.66	99.3 ± 0.77	98.8 ± 1.20	99.3 ± 0.77	97.2 ± 1.5

[a] Values are the mean of three determinations ± RSMD

TABLE 7 : Analysis of GPZ and GCZ in their tablet form using the proposed FTIR and reported methods

Product	Recovery (%± RSMD)[a]		F-value[b]	t-value[b]
	Proposed method	Reported method[4,7]		
Mindiab® tablets	98.1± 1.15	99.4± 0.69	2.52	1.40
Diamicron® tablets	99.5±1.02	100.6 ± 1.15	1.47	0.82

[a] Values are the mean of three determinations ± RSMD.; [b] Theoretical values for t and F at 95% confidence limit (n = 5) were 2.78 and 6.39, respectively.

proposed and the reported methods at 95% confidence level. This indicated similar accuracy and precision in the analysis of GPZ and GCZ in their tablet form.

CONCLUSION

The present study allowed the assignment of specific FTIR spectral features of each of the drug investigated in the light of their available single crystal structure data. The discrimination of two sulfonylurea oral hypoglycemic drugs can be reliably done. The FTIR spectroscopy has been utilized for the first time to quantify the studied drugs and their binary mixtures by integrating the peak areas of their diagnostic and well-resolved bands. Moreover, this vibrational spectroscopic technique appears to be an efficient tool to detect the active ingredients present in a complex matrix in the intact solid dosage forms.

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