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Fourier transform infrared spectroscopy for in-process inspection, counterfeit detection and quality control of anti-diabetic drugs

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Abstract. The purpose of this study is to develop simple and cost-effective Fourier transform infrared (FT-IR) methods for quality control evaluation of repaglinide (RPG), rosiglitazone maleate (RGZ), pioglitazone hydrochloride (PGZ) and metformin hydrochloride (MET) and assess their use for in-process quality control and detection of counterfeit medicine. The conventional KBr disc sampling technique used in FT-IR does not result in constant path-length thus impeding the use of this sensitive and simple technique for quantification of drugs. In this study, FT-IR quantitative assays were developed using a constant KBr disc pathlength to quantify drugs in bulk and tablets. Method validation was done according to the International Conference on Harmonization (ICH) guidelines and recovery studies were performed by applying standard addition technique. Samples representing pitfalls at different tablet manufacturing stages and counterfeit medicines were prepared and tested by FT-IR. The developed methods achieved ICH validation parameters. Statistical comparison of the results with reference or reported methods showed no significant difference with respect to method accuracy and precision. The methods were applicable to tablet dosage form with average recovery ranging between 99 and 102%. The developed methods are capable of detecting impurities that result from in-process manufacturing problems and counterfeit products and are inclusive for in-process and end product testing.

Keywords: FT-IR spectroscopy, repaglinide, rosiglitazone maleate, pioglitazone hydrochloride, metformin hydrochloride

1. Introduction

Several methods have been reported for the determination of the anti-diabetic agents RPG, RGZ, PGZ and MET. An HPLC method for the determination of RPG was described in the United Sates Pharmacopoeia (USP) [27]. Electrochemical, spectrophotometric and chromatographic methods have also been reported for the determination of RPG [5,7,11,19,20]. RGZ has been determined by first-order UV spectroscopy and chromatography [6,10,12]. The Indian and United States Pharmacopoeias both described HPLC methods for the quantitative determination of PGZ, zero-order and second-order UV, chromatographic and flow injection chemiluminescent methods have been reported for its determination [1,8,22–24,26,29]. UV spectroscopy and HPLC methods have been described by USP for determination of MET in powder and tablets, respectively [28]. Other spectophotometric and chromatographic methods have also been reported for MET determination [2,16–18].

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USP recommends the reduction of the reagent and chemicals used in pharmaceutical analysis that may impact human health and environment. The complex, expensive and time consuming nature of the previously reported methods make them less favourable for routine quality control applications. Also, such methods require additional steps for detection of counterfeit and detection of unexpected impurities that result from inefficient manufacturing strategy.

FT-IR spectroscopy is a rapid technique, requires no or minimal sample pretreatment and provides comparable accuracy to other established methods as well as insight about the presence of impurities. Using this technique, pharmaceutical samples can be scanned and averaged upto 64 times in any physical state in less than one minute at a high resolution and a high wavelength precision. Moreover, statistical analysis based on the spectral information obtained can be automatically done for the selection of the IR region (in terms of peak area, peak hight or peak ratio) most suitable for quantitative determination.

Recently, FT-IR methods have been developed for the quantitative determination of pharmaceutical active ingredients. FT-IR was used for the determination of naltrexone in bulk and tablet dosage form using the attenuated total reflectance (ATR) sampling technique and partial least square (PLS) chemometric approach wavelength selection [14]. Levodopa and carbidopa in binary solutions have been quantitatively determined by ATR–FT-IR after sample acidification [13]. It had also been applied for direct measurement of acetylsalicylic acid in different pharmaceutical products where conventional KBr-discs were used as a sampling technique; Beer–Lambert law and two chemometric approaches (PLS, PCR) were used in data processing [3]. Ibuprofen was determined by FT-IR and the method involved the extraction of the active ingredients with chloroform and measurement of area of IR band corresponding to the carbonyl group [15]. Near infrared reflectance spectroscopic method was reported for determination of MET where PLS was applied to the Savitsky–Golay smoothed first derivative spectral data revealed over wavelength range 1000–2500 nm [9]. FT-IR was successfully used for the determination of counterfeit medication (e.g., Viagra®, Cialis® and Lipitor®) [4,21,25].

This is the first report to describe the use of FT-IR for the quantitative determination of RPG, RGZ, PGZ and MET in bulk and their corresponding tablet dosage form using a modified KBr disc sampling technique. The methods were also used for assessing poor manufacturing and detection of counterfeit. The proposed methods were compared to other reference and reported methods for quantitative determination of the studied drugs.

2. Material and methods

2.1. Instrumentation

A Nicolet 380 FT-IR spectrometer, supported with OMNIC TM software for data processing and TQ-EZ software were used for quantitative measurement (Fischer, USA). A 35 mm agate mortar and pestle (Fischer, USA) and a hydraulic-press were used to prepare the KBr sample disc of RPG, RGZ, and PGZ using a Perkin Elmer die press. Thermo-scientific (Fischer, USA) Qwik handi-press instrument was used to prepare the KBr sample disc for MET.

2.2. Chemicals and reagents

Pure samples: RPG, RGZ, PGZ and MET were obtained from their manufacturing companies (Novondordisc, The Netherlands; Multiapex Pharma, Unipharma, and EvaPharma, Egypt; respectively) and their purity ranged from 99.9–100.2%. IR grade KBr was used (Fischer-Scientific, USA) to prepare

sample discs and chloroform (Sigma-Aldrich, Germany) was used for RPG extraction from its tablets. 2.0 g% (w/w) mixtures of RPG, RGZ, PGZ and MET in KBr were prepared and grinded.

Tablet samples: Novonorm® 2 mg, Rosozone® 4 mg, Diabetin® 30 mg and Glucophage® 1000 mg commercially available tablets were used for testing the applicability of the procedures on RPG, RGZ, PGZ and MET tablet dosage form, respectively.

All chemicals and reagents were stored in dry conditions throughout the experiment.

2.3. Experimental procedures

2.3.1. Disc preparation and FT-IR spectra recording

In order to adapt the conventional KBr disc sampling technique for quantitative determination, the thickness of the KBr disc must be kept constant (in order to obtain the same pathlength) for all the analyzed samples. The desired drug quantity were obtained from the 2.0 g% (w/w) drug/KBr mixtures and grinded in agate mortar for 2 min, completed to 0.15 g with KBr, then grinded again and pressed under 15,000 lbs by the hydraulic pressure system in the die-press for 3 min to obtain the RPG, RGZ and PGZ sample discs. For MET, the weight was completed to 0.07 g, grinded and pressed by the Qwik handi-press for 3 min to obtain the MET sample disc (the Glucophage® tablet is mainly composed of compressed MET powder coated with a thin film). FT-IR spectra were obtained for fused sample discs (of drugs and KBr prepared by conventional methods) against 100% KBr disc (blank). The interferograms were averaged over 32 scans to give spectra in the 400–4000 cm^{-1} range at a resolution of 4 cm^{-1}. Under these conditions, it took approximately 15 s to record a spectrum.

2.3.2. Selection of IR region for quantitative determination

For each drug, sample training sets composed of 4 different drug quantities in its pure and tablet dosage form in the range 0.1–1.0 mg were processed into sample discs and analyzed by FT-IR. The resulting spectra for each drug were analyzed by the TQ-EZ software to select the IR region (in term of peak area, peak height or peak ratio) most suitable for quantitative determination (Table 1).

2.3.3. Method validation

2.3.3.1. Linearity. From the 2.0 g% (w/w) drug/KBr mixtures the weight equivalent to 0.1–1.0 mg of each drug were processed into sample discs and FT-IR spectra were recorded. Calibration curve for each drug was prepared by plotting area of FT-IR absorbance band (measured by the TQ-EZ software) against concentration and regression equations were computed.

2.3.3.2. Accuracy. Four replicates of at least 4 different drug amounts for each drug were processed into sample discs and FT-IR spectra were recorded. The resulting spectra were quantitatively ana-

Table 1
Selected absorbance bands for the FT-IR quantitative determination of tested drugs

	RPG	RGZ (method I)	RGZ (method II)	PGZ	MET
Band location (cm^{-1})	2811.7–2796.28	1587.13–1577.49	1697.05–1689.34	1336.43–1330.64	740.53–734.75
Baseline	One point	One point	–	Two points	One point
Location of baseline (cm^{-1})	2832.92	1590.99	–	1346.07, 1324.86	746.32

Note: Area of FT-IR absorbance band was measured for all drugs.

lyzed by TQ-EZ software and regression equations were used for calculating the amount of recovered drug.

2.3.3.3. Precision (*repeatability*). For each drug, 4 different concentrations were analyzed three times each using the specified FT-IR procedures and SD and CV of the analytical response were calculated.

2.3.3.4. Limit of detection (*LOD*) *and limit of quantification* (*LOQ*). LOD and LOQ were calculated based on the standard deviation of the response and the calibration curve slope using the equations LOD = 3.3 σ/S and LOQ = 10 σ/S; where σ is the SD of the response and S is the slope.

2.3.3.5. Robustness (*effect of disc weight*). Using the 2.0 g% (w/w) stocks, weight equivalents of 0.6 mg of RPG, RGZ, PGZ and MET were completed with KBr to 0.1, 0.2, 0.3, 0.4 or 0.5 g (for each drug) except for MET weight were completed to (0.05, 0.06, 0.07, 0.08). Each sample was processed into a disc as described above, FT-IR spectrum was recorded, and the automatically selected parameters (Table 1) were measured. The SD, RSD and CV of the analytical response (area of FT-IR absorbance band) for each drug were calculated.

2.3.3.6. Application to tablets. The commercial tablets were weighed, grinded and the desired amount equivalent of each drug was mixed with KBr to prepare 2 g% (w/w) of each drug/KBr mixture (tablet stock). The same disc preparation and analysis procedure used for pure drugs were followed. For Novonorm® 2 mg (RPG) tablets, RPG was extracted by chloroform. No extraction was needed for the rest of drugs as there was no interference between the spectra of additives and drug spectra in the selected quantitative IR region. Because after extraction of RPG from tablets masking of FT-IR bands by additives did not allow proper quantitative measurements thus spectral subtraction of the additives was done for Novonorm® 2 mg (RPG). For Rosizone® 4 mg tablets (method 2); spectral subtraction was enough to remove interference from additives. Recovery study was performed by applying standard addition technique. The amounts of drug were calculated based on the regression equations computed under linearity.

2.3.4. In-process quality evaluation using FT-IR

A set of experiments were performed to investigate the feasibility of FT-IR for In-process quality testing. Random samples from all experiments were processed into sample discs and FT-IR spectra were recorded.

2.3.4.1. Mixing

2.3.4.1.1. Improper mixing. The placebo ingredients (starch, Mg stearate, lactose) were improperly mixed into a mortar (e.g., mixing a large quantity of ingredients in a small mortar or mixing ingredients for a short time).

2.3.4.1.2. Unclean container. Mixing was done using a mortar which was contaminated with tryptophan and citric acid. Placebo ingredients were then added into the mortar and mixed well with the pestle inducing a sheer effect.

2.3.4.2. Granulation

2.3.4.2.1. Granulation. Citric acid was levigated with water into the mortar for 30 min then removed and passed through a sieve. The mortar and sieve were left to dry. The placebo ingredients and drug were mixed and in the mortar and passed through the sieve.

2.3.4.2.2. Moisture. The previous procedure was repeated in a wet mortar.

2.3.4.2.3. Contamination with ethylene glycol. The procedure was repeated after mixing 0.2 μl ethylene glycol with placebo in a dry mortar.

2.3.4.3. Pressing. The quick handi-press was used to press a sample containing gelatin and placebo. Tablet was removed and the quick handi-press was used for a laboratory prepared PGZ tablet. The produced tablets were then grinded.

2.3.5. Detection of counterfeit

Counterfeit tablets were prepared including tablet with no drug (placebo), non-authentic tablet with drug, and deteriorated tablet (due to poor manufacturing, stressful storage, etc.). Placebo consists of starch, lactose and Mg stearate. RPG, PGZ and RGZ were separately added to placebo to give non-authentic tablets. Prepared tablets were mixed with 1 ml of 1 N HCl and left for one hour (stress conditions). Random samples from the experiments were processed into discs and FT-IR spectra were recorded.

3. Results

For quantitative determination of RPG, the area of FT-IR absorbance band between 2811.7 and 2796.28 cm^{-1} was employed (corresponding to C–H stretching next to amide). The baseline was set as one point at 2832.92 cm^{-1}.

For quantitative determination of RGZ two areas of the FT-IR absorbance band were automatically selected. The first area (method I) was between 1587.13 and 1577.49 cm^{-1} (corresponding to double bond of benzene ring stretching) and baseline was one point at 1590.99 cm^{-1}. The second area (method II) was between 1697.05 and 1689.34 cm^{-1} (corresponding to C=O stretching) and did not require baseline adjustment.

For PGZ the area between 1336.43 and 1330.64 cm^{-1} (corresponding to C–S stretching) and the baseline was adjusted as two points at 1346.07 and 1324.86 cm^{-1}. For MET the area between 740.53 and 734.75 cm^{-1} and the baseline was set to be one point at 746.32 cm^{-1}.

The five described methods offered good linearity between the area of the FT-IR absorbance band and concentration of each drug. The linearity range was 0.1–1.0 mg for all the drugs except for MET (0.2–1.0 mg). Parameters of the regression equations for all drugs are shown in Table 2.

In the accuracy study, the accuracy percentage (±SD) was 100.47 (±0.75) for RPG; 100.99 (±0.78) for RGZ (method I); 99.31 (±1.45) for RGZ (method II); 100.47 (±1.1) for PGZ; and 100.21 (±1.75) for MET. By performing F-test and t-test no significant difference were found between the proposed methods and reference or reported methods (Table 3). As evident from Table 4, the SD of the analytical responses was low indicating good precision of the developed methods.

Limit of detection and quantification (mg) were 0.019 and 0.056 for RPG; 0.010 and 0.030 for RGZ (method I); 0.010 and 0.031 for RGZ (method II); 0.017 and 0.051 for PGZ; and 0.012 and 0.06 for MET.

Table 2
Parameters of the regression equations

Parameter	RPG	RGZ (method I)	RGZ (method II)	PGZ	MET
Linearity range (mg)	0.1–1.0	0.1–1.0	0.1–1.0	0.1–1.0	0.2–1.0
LOD (mg)	0.019	0.01	0.01	0.017	0.012
LOQ (mg)	0.056	0.030	0.031	0.051	0.06
Slope	0.8862	1.0068	0.3253	1.9449	1.6923
Intercept	0.0271	0.0185	0.026	−0.0066	0.0395
Regression coefficient	0.9993	0.9941	0.9964	0.9935	0.9923

Table 3
Statistical comparison between the reference or reported methods and the proposed FT-IR methods* for tested drugs

	RPG*	RPG reference HPLC method [27]	RGZ (I)†,*	RGZ (II)†,*	RGZ UV spectroscopic methods [6]	PGZ*	PGZ reference HPLC method [29]	MET*	MET reference HPLC method [28]
Accuracy	100.47	99.7	100.99	99.31	100.77	100.47	99.7	100.21	100.05
N	5	4	5	5	4	5	4	5	4
SD	0.75	1.00	0.78	1.45	1.00	1.10	0.83	1.75	0.90
RSD	0.75	1.00	0.77	1.46	1.00	1.09	0.83	1.45	0.90
Variance	0.5625	1.000	0.6100	2.1000	1.0000	1.2100	0.7000	3.0600	0.8100
CV	0.0075	0.1000	0.0077	0.1500	0.0100	0.0110	0.0080	0.0170	0.0090
F-test	1.78		1.29	2.16		1.72		3.78	100.05
	(6.59)		(6.59)	(9.12)		(9.12)		(9.12)	
t-test	1.28		0.36	1.78		1.19		0.18	
	(2.37)		(2.37)	(2.37)		(2.37)		(2.37)	

Notes: For F-test and t-test, figures between parentheses represent tabulated T and F-values at $p = 0.05$. † Two methods are proposed for RGZ (I, II).

Table 4
Precision (repeatability) of FT-IR measurements of each tested drug

Concentration (mg)/disc	RPG*	SD	CV	RGZ (I)†,*	SD	CV	RGZ (II)†,*	SD	CV	PGZ*	SD	CV	MET*	SD	CV
0.3	0.300	0.005	0.017	0.347	0.003	0.009	0.122	0.001	0.0082	0.290	0.01	0.034	0.540	0.010	0.019
0.5	0.459	0.036	0.078	0.477	0.028	0.059	0.197	0.013	0.066	0.433	0.019	0.043	0.903	0.022	0.024
0.7	0.651	0.005	0.008	0.732	0.017	0.023	0.252	0.022	0.087	0.646	0.025	0.039	1.221	0.030	0.025
0.9	0.834	0.001	0.001	0.911	0.089	0.098	0.308	0.029	0.094	0.933	0.012	0.013	1.596	0.050	0.031

Notes: * Proposed FT-IR method analytical response. † Two methods are proposed for RGZ (I, II). Each experiment represents an average of 3 replicates.

In the robustness study, the same procedures for disc preparation were followed but the weight of the disc was altered each time, the FT-IR spectra of the prepared sample discs were recorded and a constant path-length was selected for the quantitative analysis using the TQ-EZ software. No change in the absorbance intensity was observed indicating that the disc weight fluctuations can be circumvented by selecting a constant path-length for the TQ-EZ software (Table 4).

The method was used to quantify drugs in tablet dosage form. The additives in the RPG tablet greatly interfered with the spectrum of pure RPG, accordingly extraction of RPG from Novonorm® 2 mg tablet was done using chloroform. The tablet extract showed some absorbance bands that did not belong to the active constituents indicating that the tablet extract still contained some of the tablet excepients. The spectrum of the excepients was computed by subtracting the average of 3 pure RPG spectra from three tablet extract spectra of the same concentration. The computed excepient extract was utilized for computing the RPG spectra by subtracting it from the tablet extract spectra. The spectra of pure RPG and computed RPG spectra are shown in Fig. 1. The same procedures were applied to the RGZ tablets (method II). The spectra of the tablet dosage form for RGZ, PGZ, and MET did not show interference at

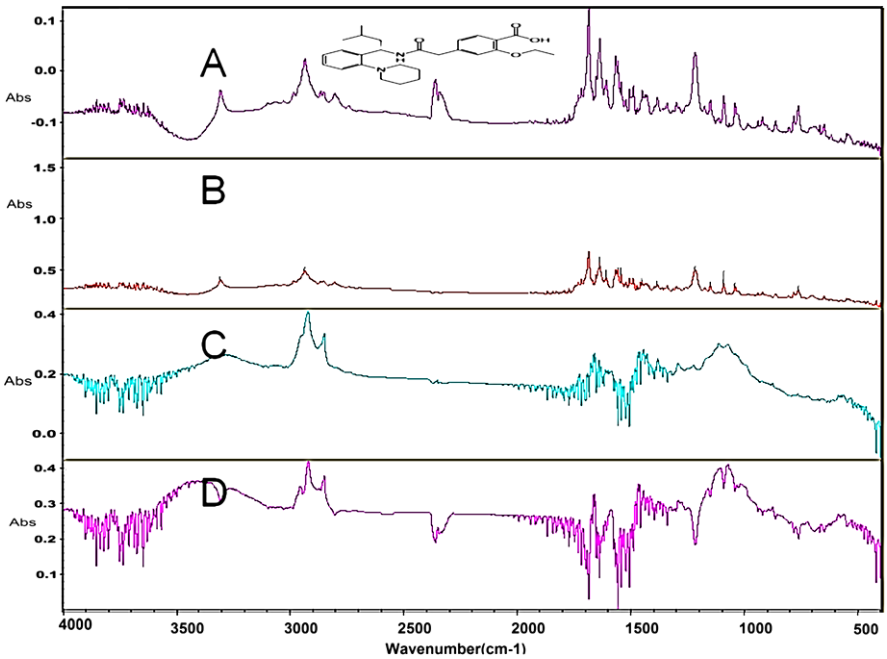

Fig. 1. FT-IR spectra of Novonorm® 2 mg tablet excipients (D) computed by subtracting pure RPG spectrum (B) from Novonorm® 2 mg tablet extract (C). (D) is subtracted from (C) to get the RPG content in Novonorm® 2 mg tablet sample (A); the RPG content in all samples was 0.2 mg. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0531.)

the selected range for quantification and are shown, together with their corresponding pure drug spectra, in Figs 2–4, respectively.

In the recovery study, the standard addition method was used where the percent recovery from tablet was 101 while that of the pure added was 100.07 (±0.976) for RPG. In case of RGZ (method I) recovery from tablet was 99% and that of the pure added was 99.98% (±0.32). When applying method II on RGZ, percent recovery of the drug from pharmaceutical product was 98.5 and that of the pure was 99.73 (±0.98). For PGZ, the percent recovery from tablet was 102 and that of the pure was 99.15 (±1.19). For MET the percent recovery from tablet was 101.86 and that of the pure drug was 100.81 (±1.17).

As shown in Fig. 5, a change in the tablet blend resulting from traces of pharmaceutical impurities due to poor manufacturing process including improper mixing, unclean conditions or presence of moisture resulted in distinct FT-IR spectra. Counterfeit tablets including tablets with no active ingredients (Fig. 6), non-authentic tablets containing active ingredients (Fig. 7) and altered active ingredients (Fig. 8) gave FT-IR spectra that are different from the generic tablets (Figs 1–4).

4. Discussion

A new FT-IR method was developed for the determination of RPG, RGZ, PGZ and MET. The conventional KBr disc sampling technique that is used for the qualitative assessment of drugs was modified for quantitative determination of pharmaceuticals. This allows for both direct evaluation of samples and gives an insight about its constituents including drug, excipients, and impurities. A key step in this

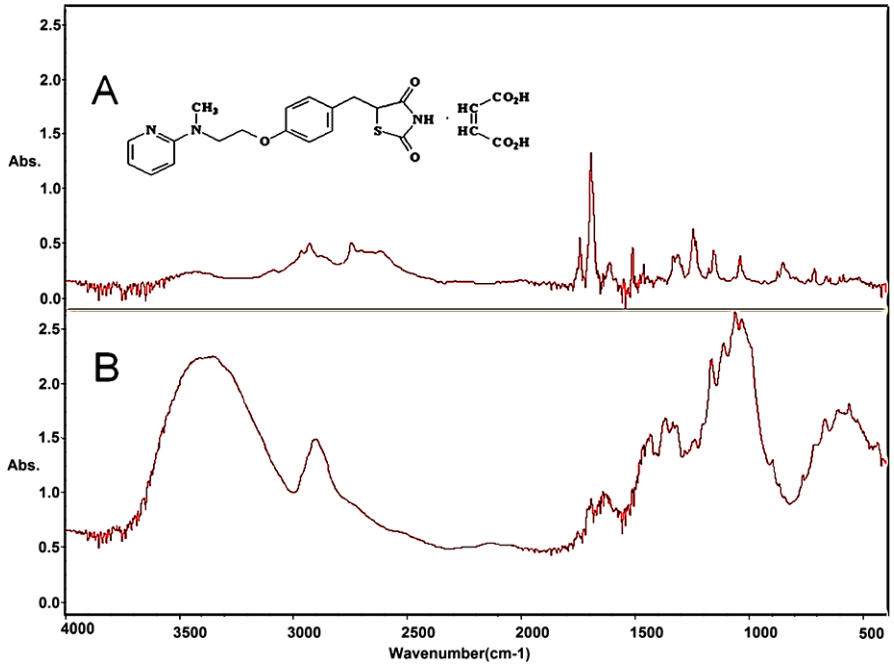

Fig. 2. FT-IR spectra of 0.2 mg pure RGZ (A) and Rosizone® 4 mg tablet (B). (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0531.)

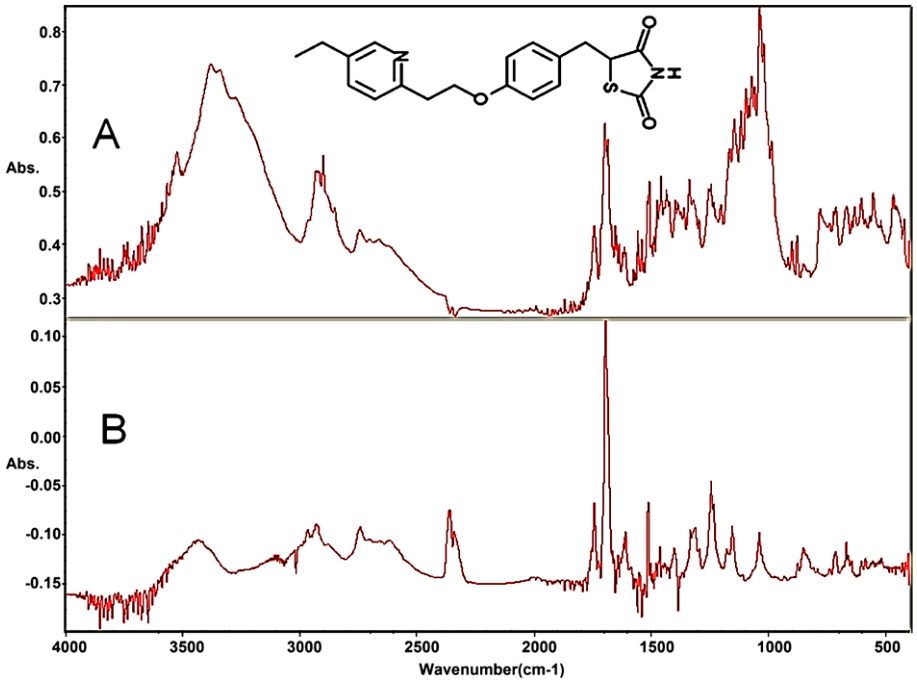

Fig. 3. FT-IR spectra of Diabetin® 30 mg tablet (A) and pure PGZ (B). (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0531.)

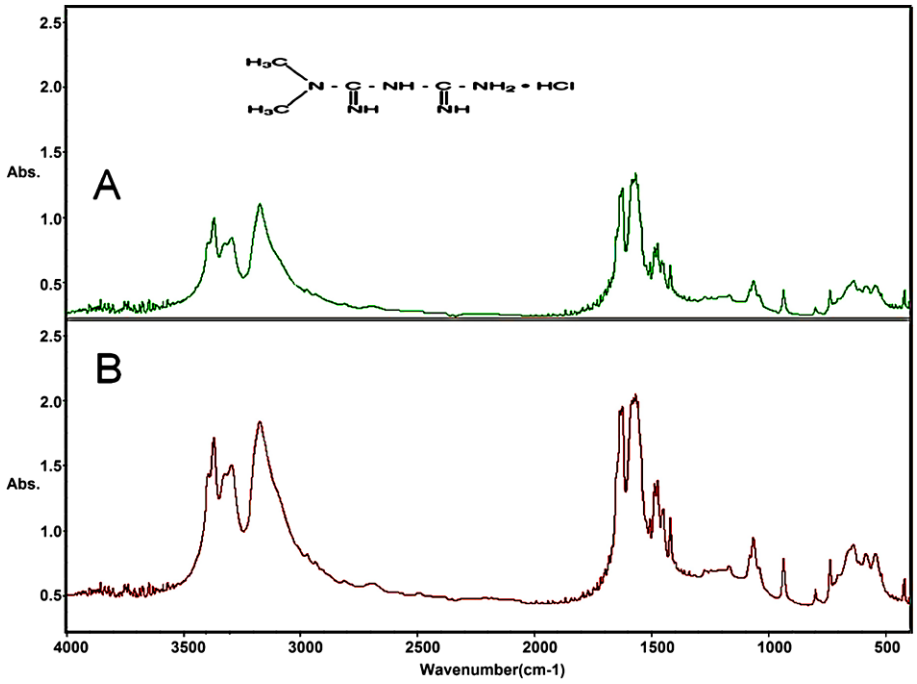

Fig. 4. FT-IR spectra of Glucophage® 1000 mg tablet (A) and pure MET (B). (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0531.)

method is to prepare KBr discs of constant pathlength. This was done by using discs of the same total weight and applying a constant pressure for the same period of time for all samples. We extended the use of the new method to cover different manufacturing stages including acceptance of bulk substances, in-process quality testing for GMP applications, end product evaluation and detection of counterfeit.

The FT-IR method was used to detect manufacturing problems that may result from non uniform tablet blend, unclean container, granulation problems, moisture, and contamination such as ethylene glycol and gelatine. FT-IR generated spectra with different patterns from expected for samples taken from the previously mentioned poor manufacturing stages. For detecting the counterfeit, placebo, non-authentic tablets (which contain the active ingredient but not the authentic tablet blend), and tablets that are subjected to 1 N HCl (to simulate stressful conditions) were tested. The generated FT-IR spectra contained distinct differences from that of the authentic tablet.

FT-IR can be used for quantitative analysis of anti-diabetic drugs in bulk and in pharmaceutical dosage form. The developed methods have comparable accuracy and precision to those of the reference and reported methods and required no or minimal sample pre-treatment. Other methods, such spectrophotometer and HPLC, require sample pre-treatments or extraction of active ingredients and do not allow detection of non-authentic tablets which contain active ingredients.

FT-IR provides an insight into the impurities that may be present in drug samples and can be used as a screening tool for counterfeit medications. Accurate quantitative determination of active ingredient is possible even in case of incomplete knowledge of dosage form formula. FT-IR allows insight into whole sample constituents and is thus suited for in-process and end product quality testing and to distinguish counterfeit from original tablets.

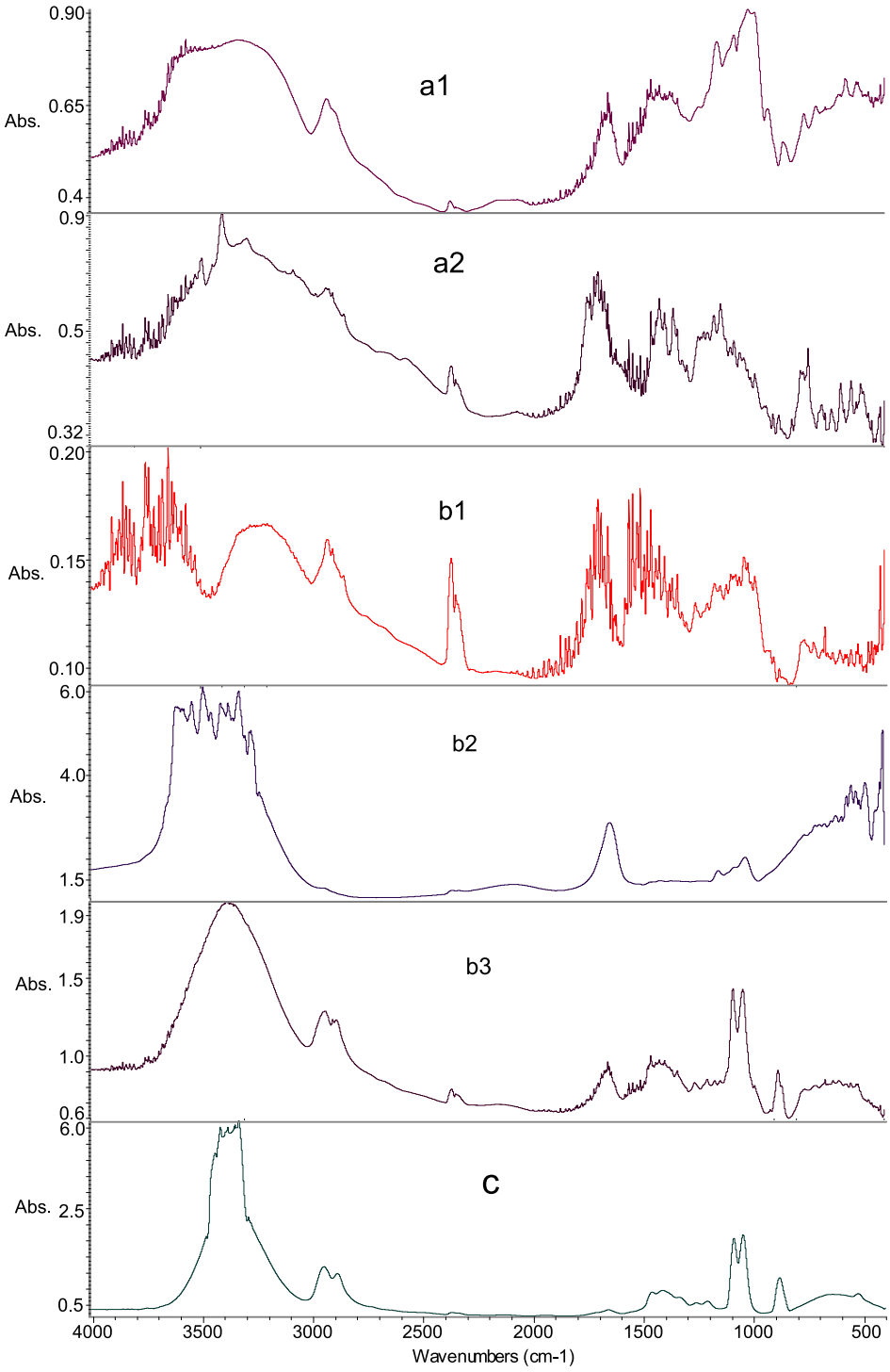

Fig. 5. FT-IR spectra for in-process quality testing in cases of: (a) problems with mixing: 1 – improper mixing, 2 – unclean mixer. (b) contamination during granulation: 1 – citric acid, 2 – moisture, 3 – ethylene glycol. (c) contamination after pressing: gelatine. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0531.)

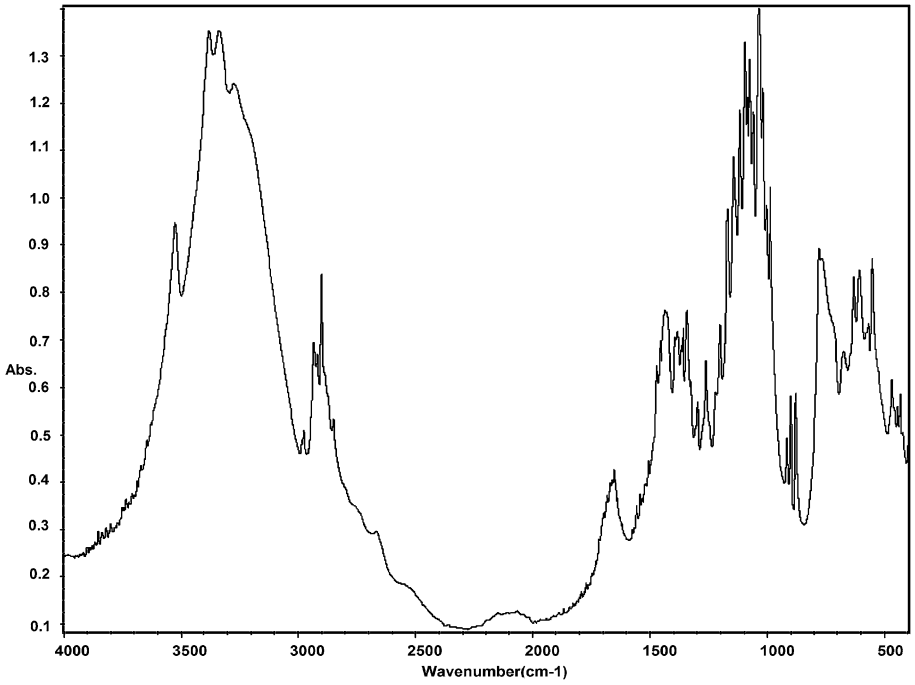

Fig. 6. FT-IR spectrum of placebo.

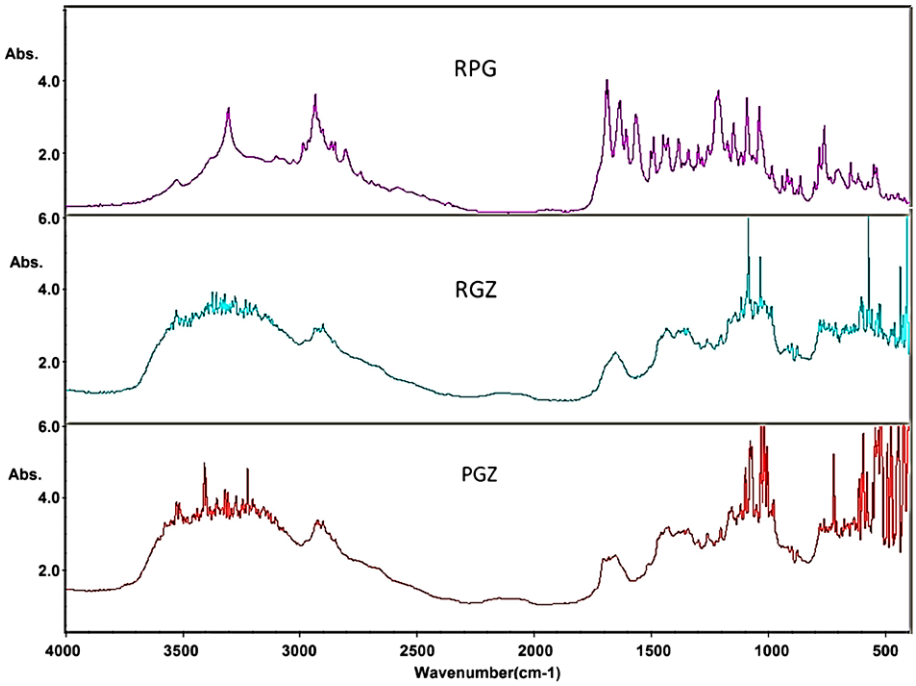

Fig. 7. FT-IR spectra of RPG, RGZ and PGZ laboratory prepared tablets. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0531.)

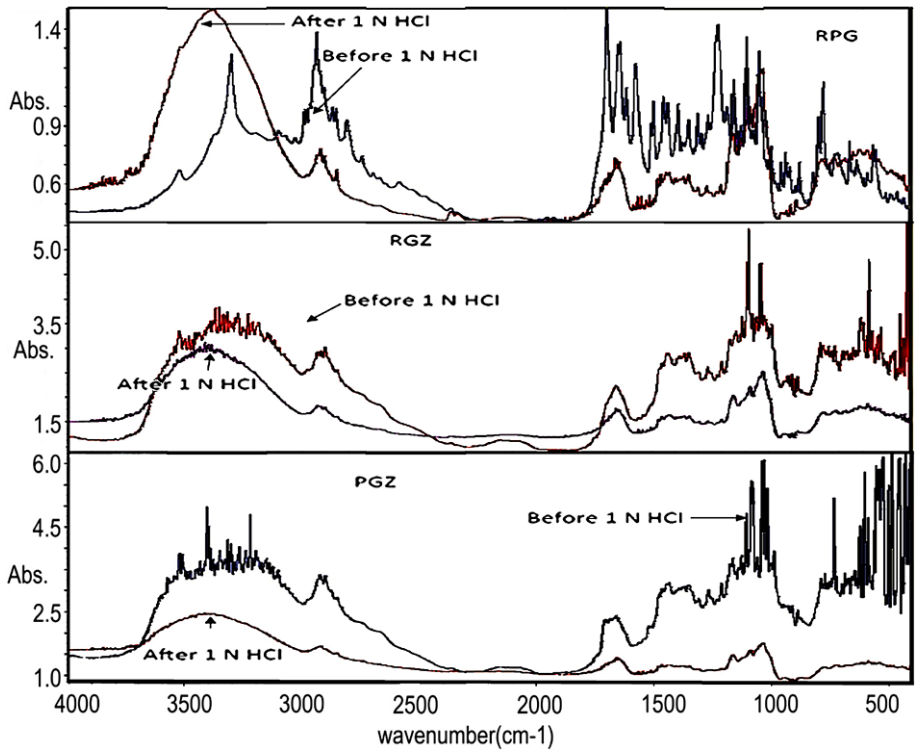

Fig. 8. Superimposition of prepared tablets before and after treatment with 1 N HCl: RPG, RGZ, PGZ. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0531.)

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