

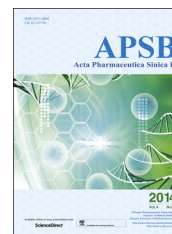
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ORIGINAL ARTICLE



Transmission FTIR derivative spectroscopy for estimation of furosemide in raw material and tablet dosage form

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Abstract A Fourier transform infrared derivative spectroscopy (FTIR-DS) method has been developed for determining furosemide (FUR) in pharmaceutical solid dosage form. The method involves the extraction of FUR from tablets with *N,N*-dimethylformamide by sonication and direct measurement in liquid phase mode using a reduced path length cell. In general, the spectra were measured in transmission mode and the equipment was configured to collect a spectrum at 4 cm^{-1} resolution and a 13 s collection time (10 scans co-added). The spectra were collected between 1400 cm^{-1} and 450 cm^{-1} . Derivative spectroscopy was used for data processing and quantitative measurement using the peak area of the second order spectrum of the major spectral band found at 1165 cm^{-1} (SO_2 stretching of FUR) with baseline correction. The method fulfilled most validation requirements in the 2 mg/mL and 20 mg/mL range, with a 0.9998 coefficient of determination obtained by simple calibration model, and a general coefficient of variation $<2\%$. The mean recovery for the proposed assay method resulted within the $(100 \pm 3)\%$ over the 80%–120% range of the target concentration. The results agree with a pharmacopoeial method and, therefore, could be considered interchangeable.

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Abbreviations: API, active pharmaceutical ingredient; DMF, *N,N*-dimethylformamide; DS, derivative spectroscopy; FTIR, Fourier transform infrared; FUR, furosemide or frusemide; HPLC, high performance liquid chromatographic; MIR, mid infrared

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1. Introduction

Furosemide or frusemide (FUR) is a loop diuretic widely used in the treatment of congestive heart failure and edema¹. As a commercial product, this active pharmaceutical ingredient (API) is administered basically as an oral tablet and an injectable solution². FUR is a potent diuretic with some associated side effects. Additionally, FUR is included in the World Anti-Doping Agency's banned drug list³. Therefore, we are always concerned about the availability of a valid and simple analytical method for FUR determination in pharmaceutical dosage forms.

According to the reviews published by Espinosa-Bosch et al.^{4,5}, a variety of analytical methods has been proposed for determining FUR in drugs. Li et al.⁶ also mention various methods for determining FUR since introducing it to the pharmaceutical market. In the present decade, several methods including the resonance Rayleigh scattering technique⁶, spectrophotometric^{7–9}, fluorescence^{10,11}, luminescence¹² and liquid chromatography^{13–16} have been proposed to quantify FUR in pharmaceutical samples.

The official methods for the determination of FUR as a bulk drug are based on titrimetry^{17–21} while the official methods for FUR determination in tablets are based on a liquid chromatographic method¹⁷ and an UV spectrophotometric method^{19,22}. However, until now, no direct Fourier transform infrared (FTIR) spectroscopy has been recommended for the quantitative determination of FUR in solid dosage forms.

Because of its inherent simplicity and availability in most pharmaceutical laboratories, FTIR is a technique which can be used in quality control laboratories not only for qualitative, but also for quantitative purposes. Since the wavenumber and the respective intensity of an absorption maximum depend on a particular group of the chemical structure of a substance, this measurement can be used to quantitatively determine a substance. Fig. 1 illustrates how rich the structure of FUR is in regards to diverse groups which absorb in the mid-IR (MIR) region.

For the past two decades, the transmission FTIR spectroscopy has undergone significant application for quantitative purposes, several in the pharmaceutical analysis field^{23–33}. As suggested above, a FTIR spectrum can be used for verifying the identity and purity of API and for detecting any spectral interference originating from the excipients. A conventional calibration curve approach is used as a method for analyzing the FTIR absorption spectrum. However, it usually requires chemometric methods in order to improve the data quality whenever applicable. Derivative spectroscopy (DS) is one of the easiest pre-processing methods. It is a useful technique for finding bands hidden in broad spectral features. Virtually, all current FTIR spectrometers generate derivative spectra by mathematical means. Consequently, an additional instrument is not required. Specialized literature concerning DS has been amply described^{34–36}.

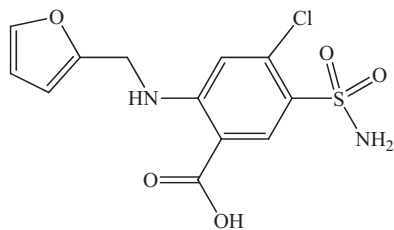


Figure 1 Chemical structure of 4-chloro-2-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid (FUR).

Therefore, this study aims to develop a new method for the qualitative and quantitative determination of FUR by FTIR-DS in liquid cell-through transmission mode. The method uses the same extraction procedure of compendium pharmacopoeias. This approach represents a real alternative for not only identifying incoming raw materials, but also analyzing them quantitatively, and for assessing pharmaceutical quality of FUR tablets from multinational markets without using separating techniques. By comparison with compendial and non-compendial analytical methods, the proposed FTIR-DS method reduces solvent consumption and eliminates the use of reagents.

2. Materials and methods

2.1. Equipment

A Perkin-Elmer Spectrum 2000 FTIR spectrophotometer (Norwalk, CT, USA) equipped with a temperature stabilized deuterated triglycine sulfate detector, a KBr-Ge coated beam splitter, and a global IR source was employed for FTIR spectra acquisition. A Wilmad Labglass FTIR liquid transmission cell (Buena, NJ, USA) was used for sampling. Its circular configuration was a general purpose liquid transmission cell. It involved ZnSe windows (32 mm diameter, 3 mm thick) and an appropriate PTFE spacer of 0.05 mm optical path length. The sample was introduced by filling ports using a 3 mL syringe.

2.2. Reagents and samples

FUR reference substance of 98.0% purity was purchased from Sigma-Aldrich (St Louis, MO, USA) and 99.05% FUR raw material purity was kindly supplied by Valmorca Lab (Mérida, Venezuela). *N,N*-Dimethylformamide (DMF) of 99.9% purity was purchased from Mallinckrodt (Paris, Kentucky, USA). Commercial pharmaceutical 20 mg/Tab and 40 mg/Tab FUR tablets were purchased from local market (Mérida, Venezuela) but were manufactured by different laboratories: Sanofi Aventis (Caracas, Venezuela), Proula (Lagunillas, Venezuela), PluxAndex (El Vigía, Venezuela), Meyer Daliol (Caracas, Venezuela) and Genéricos Rx (Caracas, Venezuela).

2.3. Preparation of a standard solution

A 40.0 mg/mL FUR stock solution was prepared in DMF and a 20.0 mg/mL diluted FUR stock solution was also prepared in DMF by diluting 1:1 (v/v). The stock solution was kept protected from light using amber colored flasks and refrigerated. After being diluted with DMF, increasing volumes of FUR stock solutions were quantitatively transferred to a set of 10 mL volumetric flasks to provide solutions within the 2.0–20.0 mg/mL concentration range. Final standard solutions were kept protected from light.

2.4. Preparation of a sample solution

Twenty tablets were accurately weighed, finely pulverized, and thoroughly mixed. An accurately weighed portion of pulverized tablets equivalent to 100 mg of FUR was transferred to a 10-mL volumetric flask, 9 mL of diluting solvent (DMF) was added, and was sonicated for 30 min. After cooling, the diluting solvent was added to bring the volume to the mark. Finally, it was mixed,

centrifuged at 2500 rpm for 5 min, and filtered, discarding the first 2.5 mL of the filtrate. All sample solutions were filtered through 0.22 μm analytical Nylon filters (New Jersey, USA). Samples for the standard addition technique were obtained by increasing volumes from 0 mL to 9 mL of the 20 mg/mL FUR stock solution to a set of 10 mL volumetric flasks containing 1.0 mL of sample solution equivalent to 20 mg/mL FUR. They were then quantitatively diluted to obtain solutions within the nominal concentration range.

2.5. Method validation

Validation of the analytical method and procedures was conducted, unless otherwise specified herein, following a tutorial from LabCompliance, a private organization devoted to validation and qualification in analytical laboratories³⁷.

3. Results and discussion

3.1. Spectral characterization of FUR in DMF

FUR is insoluble in water or in chloroform. It is freely soluble in acetone, DMF and alkali-metal hydroxide solutions. We selected DMF taking into account FUR solubility, volatility and IR transparency. This is considered a green chemical solvent due to its amenable properties, such as low toxicity, low water miscibility and low volatility³⁸.

As can be expected, in order to use DMF as a solvent in the IR spectroscopy, a reduced optical path length was required. An optical path length of 0.05 mm resulted adequate for the purpose of finding transparency zones in the MIR region. A guide to path length selection and cell thickness, depending on analyte concentration in the sample solution, has been described by Stuart³⁹. Using DMF as the background, transparency zones were found in the IR fingerprint range ($\tilde{\nu}/\text{cm}^{-1}$) as follows: 1370–1270, 1240–1120 and 1050–500 (Fig. 2).

The FTIR spectrum of FUR obtained in the solid phase, KBr pellets, shows well defined bands with relatively high intensity in the 1800–400 cm^{-1} wavenumber range. Using the KBr pellet, comparison of the FUR analyte FTIR spectrum vs. the FUR reference spectrum from the International Pharmacopoeia website²¹ showed an

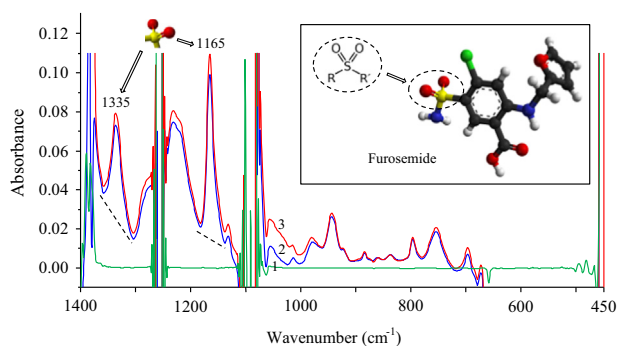


Figure 2 Liquid phase FTIR spectrum of DMF (spectrum line 1), FUR standard 10 mg/mL (spectrum line 2), and FUR pharmaceutical sample (spectrum line 3). The nominal concentration of the pharmaceutical product is equivalent to 10 mg/mL. Spectra are the average of 10 accumulated scans obtained with a nominal resolution of 4 cm^{-1} using DMF as background correction.

excellent frequency correlation. The chemical sulfur group (Fig. 1) gives specificity to the FUR chemical structure. Indeed, SO_2 and SO sulfur compound groups produced strong IR bands in the 1400–1000 cm^{-1} range. The expected S=O stretching bands for sulfonamides are as follows: 1390–1290 cm^{-1} range for SO_2 asymmetric stretching; 1190–1120 cm^{-1} range for SO_2 symmetric stretching; and 1060–1020 cm^{-1} range for S=O stretching³⁹.

Therefore, taking into account the above information, the FUR FTIR spectrum, using DMF as background, showed two notable spectral bands in the MIR with maximum wavenumbers located at 1335 cm^{-1} and 1165 cm^{-1} (Fig. 2). The spectra also showed that the presence of excipients, at the stated concentrations in the formulation, did not have any additional IR bands interfering with the mentioned IR absorption bands belonging to FUR.

3.2. Selection of the analytical spectral band

The two FUR spectral bands in the MIR, referred to above, showed a slightly spectral baseline shift for FUR in the pharmaceutical formulation. Under these circumstances, we decided to use a pre-processing method. When analyzing spectral data, our research group commonly used the concept of derivativizing spectral data, one of the most used pre-processing methods in spectroscopy. It allows for minimizing unwanted variations such as baseline shift, enhancement of spectral significant differences, and eliminating interferences by broad band constituents^{34,35}.

The DS revealed that first-order spectrum, a plot of $dA/d\lambda$ vs $1/\lambda$, allowed eliminating baseline shifts. However, apparently the FUR standard spectrum did not highly resemble the FUR sample (figure not shown). Additional spectroscopic processing, using the second-order spectrum, $d^2A/d(1/\lambda)^2$ vs $1/\lambda$, showed quite similar spectral bands for the analyte in the two analyzed solutions, sample and standard (Figs. 2 and 3).

Briefly, the negative bands of the second order derivative with minimum at the same maximum on the zero-order band were quite similar, sample and standard, coming from the two additional positive satellite bands, the first at 1174 cm^{-1} and the second at 1158 cm^{-1} . This behavior was also observed for the other possible analytical band whose maximum was found at 1335 cm^{-1} . Both signal to noise ratio and baseline shift corrections were the preponderant factors for discriminating between the two last bands. In this sense, the band at 1165 cm^{-1} provided the best precision, sensitivity, and quantitative features.

3.3. Selection of the analytical measurement criterion

Various measurement criteria were evaluated using the second-order derivative of the spectral band in the selected range (Fig. 3B). Taking into account the main features of a linear regression curve, we decided to select the area under the main second-order band denoted as D_{ZL} (1170–1158 cm^{-1}) in Table 1.

3.4. Effect of FTIR instrument conditions

A monoparametric study was performed by monitoring the selected analytical signal for each variable by triplicate. As a whole, a nominal resolution of 4 cm^{-1} , selected from 2 cm^{-1} to 32 cm^{-1} range, by accumulating 10 scans, selected from 1 scan to 50 scans, was chosen mainly as a compromise between precision of <1.0% RSD and spectral acquisition time <60 s. On the other hand, as a compromise with respect to sensitivity, precision, and

band shape, the selected derivative window for analytical purposes was 9 points, selected from a 5 points to 37 points range, with a related precision of 0.27% RSD, $n=3$.

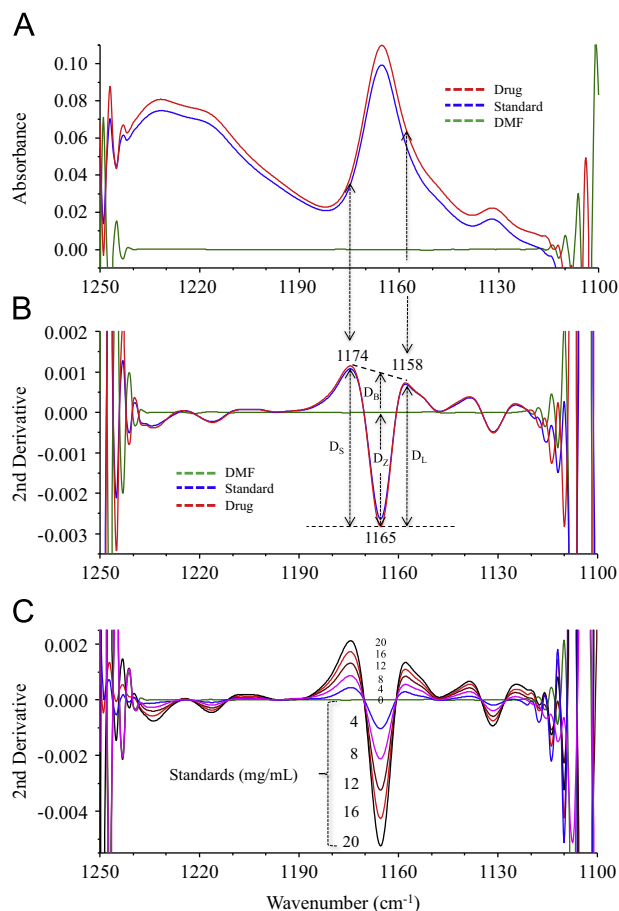


Figure 3 The zeroth (A) and second (B) derivative spectra of FUR standard and FUR sample. Second order derivative of FUR as a function of the concentration (C).

3.5. FTIR-DS system analytical figures of merit

3.5.1. Linearity

Data from the simple regression line described in Table 2 demonstrated acceptable FUR linearity over 20%–200% range of the target concentration (10 mg/mL) with a quantification limit of 1.0 mg/mL. The Y-intercept was negative but not significantly different from zero, $P=0.05$. Furthermore, the y-intercept variability resulted less than 1% of the response obtained at the target level which is usually an acceptable criterion⁴⁰. The statistical significance of the regression line ($r^2=0.9998$) also revealed that the slope is not zero, $P<0.05$, which in turn, suggested that changes in the concentration variable are highly associated with changes in the response variable (Table 2).

3.5.2. Precision

As can be seen in Table 2, data for instrument precision and repeatability met the requirements for a method to be precise³⁷.

3.5.3. Selectivity and specificity

The fingerprint region is often the most complex and confusing region to interpret. However, its utility is that many bands provide a molecule pattern. Fortunately, FUR has an inorganic group, S=O, which absorbs in the IR fingerprint region giving intense vibrational bands, fewer in number and broader than those observed for organic materials. These features generate a characteristic IR spectrum. By appropriately selecting one of these large bands, the method provided some grade of specificity. The use of DS allowed demonstrating that the selected spectral region for analytical purposes only belonged to the API (Fig. 2). Nonspecific background interference and baseline shift were automatically corrected by the conversion of the zero-order derivative spectrum into the second-order derivative spectrum, thus improving accuracy of quantification.

The standard addition technique did not evidence spectral interference due to concomitant absorbing species coming from excipients. It is important to remember that the analyte is extracted from the excipient using an organic solvent, where FUR is very soluble. This cannot be said for the known common excipients

Table 1 Analytical figures obtained for FUR determination by FTIR-DS using different measurement modes.

Band parameters (cm ⁻¹) ^a			Linear regression ^b		Analytical parameters ^c		
Criterion	Baseline mode	Peak location	$Y=a+b[X]$		r	LOQ	RSD
			$a (\times 10^{-5})$	$b (\times 10^{-3})$			
Peak area	D_{SL}	1165	15.4	2.52	0.9996	2.00	0.50
	D_{SZ}	1165	15.6	2.01	0.9995	2.12	0.72
	D_{ZL}	1165	-10.6	1.68	0.9999	1.03	0.32
	D_Z	1165	-6.0	1.42	0.9999	1.14	0.60
Peak height	D_S	1174	1.61	0.105	0.9998	1.46	1.38
	D_B	1165	-0.41	0.347	0.9998	1.44	1.45
	D_L	1158	2.63	0.067	0.9997	1.56	1.55
	D_Z	1165	-2.11	0.254	0.9999	1.05	1.90

^aFig. 3B illustrates the different possibilities to obtain the intensity of the derivative spectral band; D_L is the long wavenumber peak satellite, D_S is the short wavenumber peak satellite, and D_B and D_Z are the peak tangent baseline and the peak-derivative zero, respectively.

^b $Y=2nd$ derivative signal; $X=FUR$ concentration ranging from 2.0 mg/mL to 20.0 mg/mL ($n=10$).

^cLOQ, limit of quantification in mg/mL calculated as $10\sigma/slope$; RSD, relative standard deviation established at the target concentration; r =correlation coefficient.

Table 2 Analytical performance characteristics and system suitability of the proposed FTIR-DS method for determining FUR.

Parameter	Result	Observation
Dynamic linear range (mg/mL)	1.0–40.0	20–40 mg (Tab)
Working linear range (mg/mL)	2.0–20.0	–
Detector linearity (r^2)	0.9998	Criterion: > 0.9990
External linear regression	$Y = -1.1 \times 10^{-5} + 1.68 \times 10^{-3} [X]$	$Y = D^2$ – peak area; $X =$ concentration
SD of the response	0.00017	σ
Limit of quantification (mg/mL)	1.03	$(10\sigma)/\text{slope}$
Correlation coefficient	0.99988	Criterion: ≥ 0.999
Statistical significance of Y-intercept ($P < 0.05$)	$-0.90 < 2.36$	$\mu_\alpha = 0$; $t_{\text{Exp}} < t_{\text{Tab}}; t_{n-2}$
Statistical significance of slope ($P < 0.05$)	$175 > 2.36$	$\mu_\beta = 0$; $t_{\text{Exp}} < t_{\text{Tab}}; t_{n-2}$
Instrument precision data from 10 replicate determinations	0.23%	Acceptance criteria: $\leq 1.0\%$ (RSD)
Instrument precision data from 6 replicate determinations	0.31%	Acceptance criteria: $\leq 2.0\%$ (RSD)
Intra-assay precision by analyzing aliquots of a homogeneous sample, independently prepared	0.45% for 8 mg/mL ($n=3$)	Acceptance criteria: $\leq 2.0\%$ (RSD)
	0.30% for 10 mg/mL ($n=3$)	
	0.38% for 12 mg/mL ($n=3$)	
Intermediate precision:		Acceptance criteria: 2.0–5.0% (RSD)
Operator 1, day 1 and day 2	0.83%	
Operator 2, day 1 and day 2	1.6%	
Operators, day 1	1.3%	
Operators, day 2	1.2%	
Analytical frequency (sample/h)	25	Manual sampling
Stability of analytical solutions (coefficient of variation)	1.2% ($n=13$)	Cell removed after each measurement
Time extraction during sonication depending on FUR concentration in sample to be prepared	Test period: 10–120 min.	1 mg/mL
	< 10 min	10 mg/mL
	< 15 min	40 mg/mL
	< 70 min	
Sample carry over by filling the cell with a high standard concentration	0.16% (followed by a blank solvent injection)	Acceptance criteria: < 2.0% (RSD)

Standard deviation, SD. Relative standard deviation, RSD. Symbol “ σ ” represents $SD_{(Y/X)}$. Tablets, Tab. Second order derivative, D^2 .

such as maize starch, talc, magnesium stearate, sodium starch glycolate, and colloidal silicon dioxide, except for lactose, which is very soluble in DMF². Preliminary studies showed a lactose transmission FTIR spectrum with absorption intensity values in the 1150–950 cm^{-1} region, where solvent absorption saturation was observed. The proximity of lactose absorption, characteristic IR absorption of carbohydrate moieties, to FUR absorption only caused displacement of the baseline (1158–1000 cm^{-1}). Depending on the pharmaceutical brand type, displacement was caused in one more than the other (Fig. 2). This spectral interference was corrected later on using second order DS.

Furthermore, as can be seen in Figs. 3 and 4, supplementary studies demonstrated the viability of the intended objective of the analytical procedure. The visual spectral evaluation showed similar band shapes for all the commercial samples. Also, they were quite similar to the standards. Certainly, at the level of the satellite bands of the second derivative spectra, some spectral differences were observed from sample to sample. Nevertheless, they were attributed to small variations in concentration (Fig. 3C) more than any spectral interference from the excipients (Fig. 4).

3.5.4. System suitability

Generalizing, Table 2 shows the analytical performance characteristics of the FTIR-DS method for determining FUR. In this

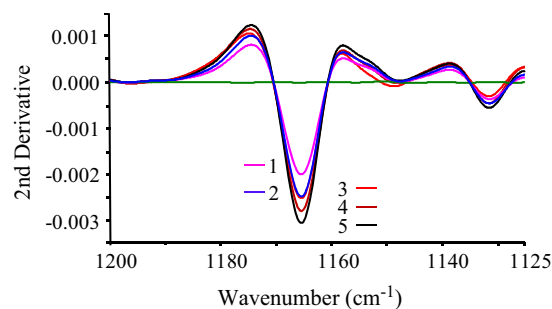


Figure 4 Representative FUR spectra in various commercial brands and a FUR standard as reference. FUR standard, 8 mg/mL (spectrum line 1), generic drug 1 (spectrum line 2), innovator drug (spectrum line 3), brand drug 1 (spectrum line 4), and generic drug 2 (spectrum line 5). Nominal concentration of pharmaceuticals represents 10 mg/mL FUR.

particular case, the table also shows some required analysis factors used in order to guarantee system suitability, such as stability of the sample solutions and percentage of extraction by sonication. This last procedure, using DMF, is official in most compendium pharmacopoeias. Although, in a lengthy procedure, several samples can be extracted simultaneously, even during the pre-conditioning

of the analytical equipment. Variation coefficients within an acceptable range demonstrated that factors, which could affect robustness of the method, had a negligible effect on the analytical response (Table 2). Other parameters were determined *via* robustness (Section 3.5.5).

3.5.5. Robustness

In principle, the proposed method could be considered as very simple. Consequently, it involved few parameters that could affect the method performance. FTIR analytical procedures are usually protected from changes in humidity and temperature. Therefore, they were kept controlled throughout the methodological development. The sample was introduced *via* filling ports. In this regard, signal response variation due to cuvette manipulation was kept to a minimum by prolonging sample application on the entrance port. That is, the sample solution was passed through the cell interior for at least 30 s at 0.5 mL/min. Additionally, when the cell was filled with a high standard concentration, followed by a blank solvent injection, the variation coefficient due to sample carry-over was very low (Table 2). Parameters such as pH and composition of the dissolvent were not involved in the method development. Possible DMF water content was corrected by the background and the selected mode of quantification, *i.e.* by DS. In conclusion, the analytical conditions can be adequately controlled and small changes in method parameters will show negligible effects on the analytical response.

3.5.6. Accuracy

Samples spiked with known quantities of the API were analyzed. The standard addition calibration test of significance for the innovator product showed that the estimated y -intercept was different from zero ($P < 0.05$) using $H_0: \mu_\alpha = 0$ ($t_{\text{Exp}} < t_{\text{Tab}}$), the result being $27 > 2.36$. A similar result was observed for the y -intercept standard addition calibration for one of the representative generic products, $20 > 2.36$. On the other hand, the standard addition slope belonging to the innovator product was within the confidence limits of the slope of reference ($1.68 \times 10^{-3} \pm 2.3 \times 10^{-4}$) at the 0.05 level of significance. An identical result was observed for the standard addition slope of one representative generic product (Table 3). The relative percentage deviation between the reference slope (b) belonging to the external calibration and the other two slopes, ($b1$) and ($b2$), was less than 1.2%. This indicated that the sensitivity of the analyte was only slightly affected by the presence of any interference coming from the inactive substance formulated alongside the API.

Furthermore, assay percentage recovery of known added amounts of API in the pharmaceutical sample was also explored (Table 4). The mean recovery for the proposed assay method resulted within $(100 \pm 3)\%$ over the 80%–120% range of the target concentration for the innovator and generic pharmaceutical products. Considering that the expected recovery depends on the sample matrix, the sample processing procedure and the analyte concentration, the accuracy of the proposed method can be considered suitable for determining FUR content in solid dosage pharmaceutical products.

Additionally, an official reference assay method was used to estimate the accuracy of the proposed method. In the global F test

Table 3 Results of assessment of the linearity of the FTIR-DS system for the assay method of FUR employing the addition standard technique.

Curve type	Linear regression: $Y = a + b [X]^a$			
	$a \pm \text{SD}; (\times 10^{-3})$	$b \pm \text{SD}; (\times 10^{-3})$	r	$\text{SD}_{(y/x)}$
External (Reference standard)	-0.11 ± 0.11	1.68 ± 0.0096	0.99988	0.00017
Standard addition 1 (Innovator)	3.78 ± 0.14	1.70 ± 0.014	0.99976	0.00024
Standard addition 2 (Generic)	3.98 ± 0.20	1.70 ± 0.021	0.99949	0.00034

^aCalibration, where Y =peak area of the 2nd derivative signal; X =FUR concentration in mg/mL ($n=9$); SD, standard deviation; r =correlation coefficient.

Table 4 Recovery of FUR from pharmaceutical samples with known concentration using the proposed FTIR-DS method.

Type of brand	Sample No.	Concentration of FUR (mg/mL); $n=3$			Recovery (%)	Recovery (%)	
		Nominal	Added	Found		Mean	Acceptable ^a
Innovator	1	2.21	4.0	6.40	104.8	102.3 ± 1.9	97–103
	2		8.0	10.41	102.6		
	3		10.0	12.59	102.8		
	4		12.0	14.16	99.6		
	5		18.0	20.50	101.6		
Generic	1	2.39	4.0	6.58	104.8	101.2 ± 2.4	97–103
	2		8.0	10.50	101.4		
	3		10.0	12.26	98.8		
	4		12.0	14.28	99.1		
	5		18.0	20.72	101.9		

^aThe acceptable recovery is for the analysis of 1% (w/v) active ingredient³⁷. The accuracy criteria for an assay method (FDA) is that the mean recovery will be $(100 \pm 2)\%$ at each concentration over the range of 80%–120% of the target concentration⁴⁰.

Table 5 Analytical application of the proposed FTIR-DS method for determining FUR in solid dosage pharmaceutical products.

Type of branded drug	Concentration (mg/Tab) \pm SD; $n=6$			
	Labeled	Found	Reference HPLC method ^a	
			Found	Accepted range
		FTIR-DS method		
Innovator 1	20	20.9 \pm 0.30	19.0 \pm 0.10	18–22
Generic 1	40	39.9 \pm 0.31	40.2 \pm 0.56	36–44
Brand 1	40	41.3 \pm 0.10	41.6 \pm 0.60	36–44
Brand 2	40	40.8 \pm 0.20	39.69 \pm 0.77	36–44
Innovator 2	40	41.9 \pm 0.70	–	36–44
Generic 2	40	44.9 \pm 0.31	–	36–44
Generic 3	40	39.6 \pm 0.20	–	36–44
Bulk ^b	99.05	99.0 \pm 0.26 ^b	–	98–101

^aUSP¹⁷. The symbol (–) means that it was not analyzed by the method of reference.

^bIt represents dried substance or raw material powder. USP acceptance range in % w/w¹⁷.

from the ANOVA table, we obtained a *F*-ratio equal to 0.036 and a *P* value greater than 0.05. Therefore, we concluded that there was insufficient evidence to assume any bias between the methods at the 95% confidence level (Table 5).

4. Conclusions

The developed method for determining FUR based on the application of transmission FTIR in the liquid phase fulfilled most validation requirements in a range of concentrations suitable for quality control of both incoming raw materials and solid dosage forms (Tables 2 and 5). Therefore, this is a pharmaceutical industry quality control method that could be considered interchangeable with other pharmacopoeial methods. As referenced in practically all compendium pharmacopoeias, the major use of FTIR in the transmission mode is for the identification of API in raw materials and finished products. Regardless of the many advantages described in the last two decades²⁸, FTIR equipment in the pharmaceutical field for qualitative issues is widely available but still undervalued for quantitative purposes. We believe that FTIR instruments in conjunction with DS are ideal tools for pharmaceutical quality control. By comparison with compendial and non-compendial analytical methods, the proposed FTIR-DS method reduces solvent consumption and eliminates the use of reagents. Additionally, the proposed FTIR-DS method allows off-site re-analysis of spectra for other chemicals not originally targeted. Finally, by selecting different measurement spectral bands, this study opens up the possibility of applying the proposed FTIR-DS method to quantify FUR when combined with other API in the same dosage form.

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