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Determination of binary polymorphic mixtures of fluconazole using near infrared spectroscopy and X-ray powder diffraction: A comparative study based on the pre-validation stage results

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ABSTRACT

The aim of the present study was to develop near infrared (NIR) and X-ray powder diffraction methods (XRPD) able to determine pure crystalline form II of fluconazole in a binary polymorphic mixtures containing forms II and III. In order to give a first performance estimation of both methods, these latters were pre-validated using accuracy profiles, a statistical approach based on β-expectation tolerance intervals. Both methods showed a good trueness, precision and accuracy and their β-expectation tolerance intervals were fully included within the acceptance limits.

The comparative study was carried out using statistical analysis based on the work of Bland and Altman. A good agreement between the two methods was demonstrated indicating the interchangeability of NIR method with XRPD method.

1. Introduction

Nowadays, polymorphism determination in pharmaceutical solid drug substance has become a major matter of concern for the Pharmaceutical Industry as a proper knowledge of the crystalline transformations is requested by the regulatory authorities. According to polymorphic form, physical and chemical properties of drug (e.g. melting point, solubility, dissolution rate, chemical reaction and resistance to degradation) may be potentially different and affect its bioavailability, process-ability, and chemical and physical stability. Therefore, it is crucial to identify the optimal polymorphic form during the early R&D stages.

FDA's Process Analytical Technology (PAT) is "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring the final product quality". In a PAT context, regulatory authorities have established the need to control polymorphic forms used as drugs in order to ensure the integrity of the targeted polymorphic form during the manufacturing process and storage [1–5]. Based on its advantages such as suppression of samples preparation and of destruction, fast data acquisition and interfacing with manufacturing processes using probes, NIR spectroscopy matches the requirements of PAT.

Fluconazole (Fig. 1) is a synthetic antifungal agent belonging to the group of triazoles. It is effective in the treatment of superficial and systemic mycoses, namely in the treatment of oropharyngeal, oesophageal, and vulvovaginal candidiasis for patients with the acquired immunodeficiency syndrome (AIDS) [6–9].

The existence of three polymorphic forms of fluconazole has been reported and designated as forms I, II and III [10]. But the crystalline form of the drug substance marketed by Pfizer corresponds to form III [10]. Alkhamis et al. reported that polymorphic form II is a metastable form that is converted to the more stable form: polymorph III under the effect of compression or during the storage in standard ambience conditions of temperature and humidity [11]. At the moment pure polymorph II and III, blends of forms II and III, and monohydrate form can be found on the market [12]. These forms were characterized by X-ray powder diffraction (XRPD), thermal analysis, FT-mid-infrared and FT-Raman spectroscopy [12–16]. Only XRPD was used as quantitative method for determination

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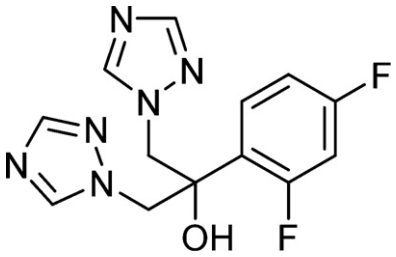

Fig. 1. Structural formula of fluconazole.

of form II in form III in binary mixtures [13]. On the other hand, Bourichi et al. demonstrated the relationship between the presence of specific impurities and polymorphic form [17].

Analytical method pre-validation is the first evaluation step of the method accuracy and it helps the analyst to optimally design the validation experiments. In previous works, accuracy profiles, a statistical approach based on β-expectation tolerance intervals was found to be advantageous to evaluate quantitative method accuracy in pre-validation and validation stages [18–20].

In a full accordance with the PAT framework, using fluconazole as the model raw material, the aims of the present paper are firstly to develop a fast near infrared method (NIR) for the determination of pure crystalline form II in binary polymorphic mixtures containing forms II and III and secondly to compare its results with those obtained with an XRPD method in a pre-validation stage.

2. Experimental

2.1. Materials

Polymorphic forms II and III of fluconazole were supplied by Vorin Laboratories Limited (Andrea Pradesh, India) and Pfizer (Dublin, Ireland), respectively. The purity of both forms was greater than 99%. The moisture content of forms II and III were 0.46% and 0.31%, respectively.

All the samples consisted in very fine white powder and were delivered in sealed containers at room temperature. The experimental protocol was carried out under controlled relative humidity and temperature conditions.

2.2. Preparation of calibration mixtures

Laboratory scale samples (150 mg total) containing various amounts of the two polymorphs were obtained by mixing known quantities of pure polymorphs II and III. The form II in form III concentration range was investigated from 0 to 100% (w/w) using samples at 25% intervals. Each sample, which was carried out in triplicate during 3 days, was mixed gently with an agate pestle and a mortar. The mixing was performed until the spectral deviation between two consecutive NIR spectra was below a fixed limit (data not shown). A total of 45 samples were used to build the calibration model.

2.3. Preparation of test mixtures

A set of 12 samples covering a concentration range from 37.5 to 87.5% (37.5, 50, 62.5, 87.5% (w/w)) was used to test the NIR method.

2.4. Reference method

The reference values used for the calibration and test samples were calculated gravimetrically from the actual weights of pure crystalline forms II and III of fluconazole in the mixtures.

2.5. Near infrared spectroscopy

NIR spectra of samples put in vials were recorded in reflexion mode using a multipurpose analyzer Fourier transform near infrared spectrometer (MPA, Bruker Optics, Ettlingen, Germany) equipped with a semi-conductor room temperature sulfide lead (RT-PbS) detector. Each spectrum was the average of 32 scans and the resolution was 8 cm^{-1} over the range from 12,500 to 4000 cm^{-1}. The spectra were collected with OPUS software 6.5 (Bruker Optics).

2.6. X-ray powder diffraction

The X-ray powder diffractograms of the samples were recorded between 5° and 35° (2θ), at room temperature, using a D8 Advance Bruker AXS spectrometer fitted with an ultrafast detector LynxEye and a copper anticathode (θ = 1.5406 Å, 40 kV, 40 mA). The diffractograms were recorded with a step width of 0.017° (2θ) and a count time of 7,8 s per step.

The quantitative determination was conducted on the integrated intensity of the peak located at about 10° (2θ), which is specific to fluconazole form II [12,13]. The composition of samples containing various percentages of pure polymorphs II and III was determined using the calibration plot of the change in the integrated 10° (2θ) peak area, as a function of polymorph II concentration.

2.7. Multivariate data analysis

PLS regression and pre-processing techniques were performed using OPUS/Quant software 6.5. All the spectral data were mean-centered prior to running the PLS.

The pre-validation of both methods was processed using the e.noval V3.0 (Arlenda, Liège, Belgium).

2.8. Agreement between NIR and XRPD

The agreement between the two methods was evaluated by a statistical analysis based on the work of Bland and Altman [21]. A plot of the relative differences between the two methods results against their average is used to compare the NIR and XRPD techniques. This comparison was performed using three samples for each concentration level corresponding to 25, 50, 75 and 100% of fluconazole during three different runs thus leading to a total of 36 results for each method. The aim of this direct method comparison is used to evaluate whether the two analytical techniques can be interchangeable. A linear mixed model was thus fitted on the results differences versus their average together with its 95% prediction interval. The computations were performed using nlme package of R v2.9.1 (CRAN, http://cran.r-project.org). The maximum relative difference that was defined acceptable between any result of the NIRS and XRPD methods was set at ±5%.

3. Results and discussion

3.1. Quantification by NIR method

The NIR spectra of pure crystalline forms II and III of fluconazole are depicted in Fig. 2. These spectra were pre-processed with a Satvisky-Golay first derivative in order to highlight the spectral differences according to the form.

Several calibration models based on PLS regression were built using different spectral ranges and common pre-processing techniques of near infrared spectral data such as spectral derivatives (Savitzky-Golay derivation) and scatter-correction methods (standard normal variate and multiplicative scatter correction) [22]. Based on the number of components in the mixtures, which are

Table 1
Spectral range, spectral pretreatment, number of latent variables, RMSEC, RMSECV and RMSEP of the NIR models.

Model	Pretreatment	Spectral range (cm^{-1})	Latent variables	RMSEC (%)	RMSECV (%)	RMSEP (%)
(a)	Raw data	9200–6500 5930–4000	2	3.77	3.99	3.48
(b)	MSC	9200–6500 5930–4000	2	1.07	1.18	0.966
(c)	First derivative + MSC	9200–6500	2	0.458	0.578	0.538
(d)	SNV	8005–4915	2	0.729	0.750	0.800
(e)	First derivative + SNV	8005–5930	2	0.828	0.847	0.697

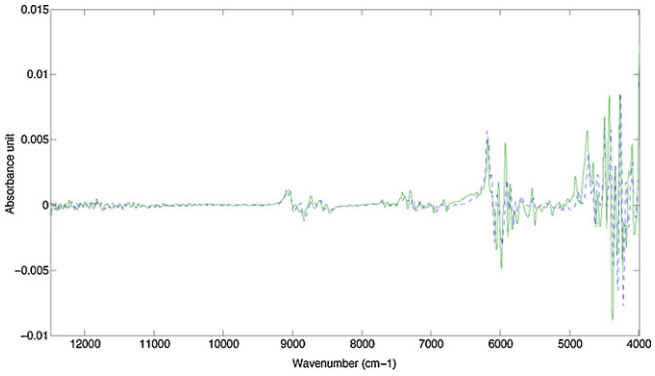

Fig. 2. NIR spectra of fluconazole: (a) form II and (b) form III.

present in the calibration set, the number of PLS latent variables of each calibration model was set to 2. Table 1 displays the parameters of the predictive models. The best results were obtained when the spectral data was first derivative prior to MSC leading to RMSEC and RMSEP values of 0.458 and 0.538, respectively.

3.2. Quantification by XRPD method

The X-ray diffraction patterns are shown in Fig. 3 between 5° and 35° (2θ). The main diffraction peaks of crystalline form III are observed at $2\theta = 11.6°$, 14.7°, 17.3°, 18.4°, 19.7°, 24.3° and 26.8°. hose of form II are observed at $2\theta = 10.0°$, 15.0°, 16.0°, 16.6°, 20.0° and 25.6°. Comparison of pure forms II and III diffractograms showed that form II has a specific diffraction peak at 10° (2θ) and thus, this last one was used for the quantification of form II in the samples of fluconazole.

3.3. Pre-validation of both methods

In this study, pre-validation experiments were carried out exclusively with the calibration set in order to give a first estimation of the performance of both methods regarding their purpose. Fig. 4 shows accuracy profiles obtained with NIR and XRPD using the calibration model (C) and a linear regression model, respectively. Due to the objective of both methods, the acceptance limits were set at ±5% and the maximum risk ($1 - \beta$) of having a future result outside these acceptance limits was set at 5%. NIRS and XRPD accuracy profiles were built based on results obtained from the leave-one-out cross-validation of the predictive model (C) and based on back-calculated results of the linear regression model, respectively. Table 2 shows the pre-validation results of both methods according to the ICH Q2(R1) validation criteria. The trueness and the precision of both methods are very satisfactory irrespective of the concentration levels and never exceed 1% and 2%, respectively.

From Fig. 4, it can be observed that β-expectation tolerance intervals of both methods are fully included within the acceptance limits of ±5%. These results gives a first guarantee that the methods should be able to achieve their objective prior running a formally validation process which is usually time-consuming regarding the use of a reference method to determine the forms II/III ratio of real samples.

3.4. Agreement between NIR and XRPD methods

The agreement between the two techniques was evaluated to know if the NIR could replace the XRPD using a methodology adapted from Bland and Altman [21]. The difference plot is shown in Fig. 5. This plot represents the relative differences of the two meth-

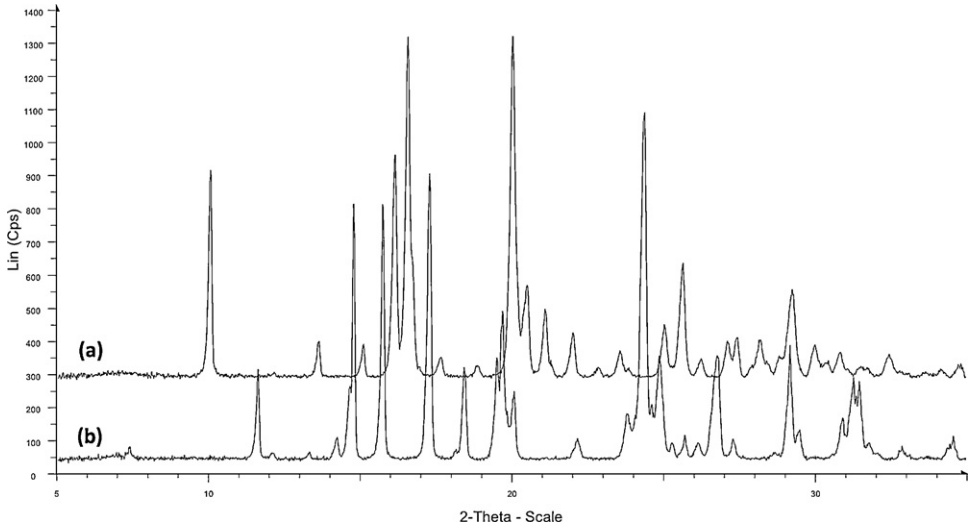

Fig. 3. Accuracy profile of fluconazole (form II) using cross validated data of PLS model (x). The plain line is the relative bias, the dashed lines are the β-expectations tolerance limits ($\beta = 95\%$) and the dotted lines represent the acceptance limits (±5%).

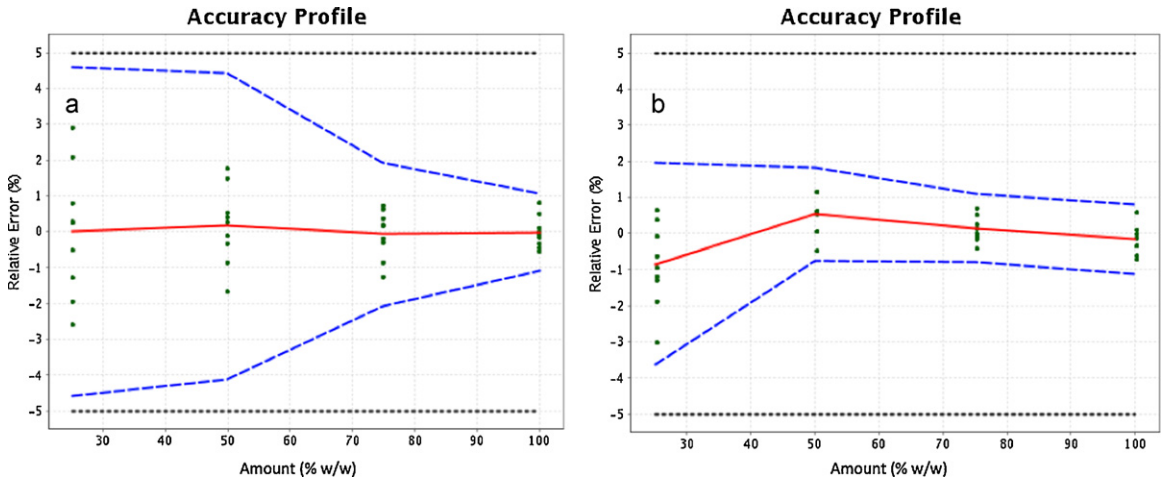

Fig. 4. RX spectra of fluconazole: (a) form II and (b) form III.

Table 2
ICH Q2(R1) validation criteria of NIR and XRPD methods.

Validation criteria			
	Concentration level (% of fluconazole II)	Relative bias (%)	
		NIR	XRPD
Trueness	25	0	−0.8
	50	0.2	0.5
	75	−0.1	−0.1
	100	0	−0.2

	Concentration level (% of fluconazole II)	Repeatability (RSD %)		Intermediate precision (RSD %)	
		NIR	XRPD	NIR	XRPD
Precision	25	1.7	1.1	1.8	1.1
	50	0.6	0.5	1.2	0.5
	75	0.5	0.3	0.7	0.4
	100	0.4	0.4	0.4	0.4

	Concentration level (% of fluconazole II)	Relative β-expectation tolerance limits (%)	
		NIR	XRPD
Accuracy	25	[−4.6,4.6]	[−3.6,2.0]
	50	[−4.1,4.4]	[−0.8,1.8]
	75	[−2.1,1.9]	[−0.8,1.1]
	100	[−1.1,1.1]	[−1.1,0.8]

	Lower LOQ (% of fluconazole II)		Upper LOQ (% of fluconazole II)	
	NIR	XRPD	NIR	XRPD
Limits of quantification (LOQ)	25		100	

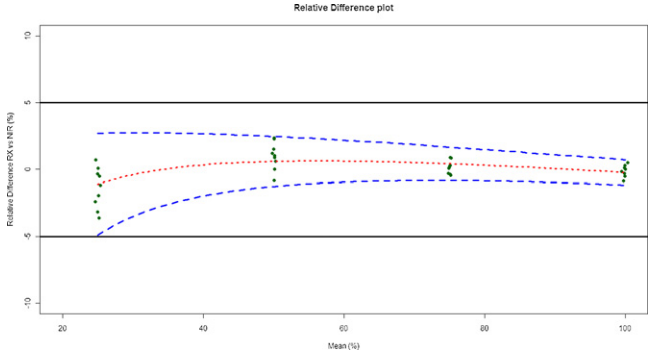

Fig. 5. Accuracy profile of fluconazole (form II) using a linear regression model. The plain line is the relative bias, the dashed lines are the β-expectations tolerance limits (β = 95%) and the dotted lines represent the acceptance limits (±5%).

ods results against their average and displays their agreement. The prediction interval limits delimits the area containing 95% of the difference values obtained. As these limits are confined inside the ±5% acceptance limits, the two methods agree sufficiently for the NIRS to replace safely the XRPD method and vice versa. Additionally it can be seen in Fig. 5 that on average the results obtained by both methods agree extremely well except for the smallest concentration level (25%) where the XRPD results under estimates systematically those of the NIRS method. However, as shown in Fig. 5, this does not impair the excellent agreement of both methods results.

4. Conclusion

NIR and XRPD methods were developed for determination of pure crystalline form II of fluconazole in binary polymorphic mix-

ture containing form II and form III. Both methods were successfully pre-validated and showed a good trueness, precision and accuracy for the determination of compound of interest irrespective of the concentration levels confirming their potential in this topic.

The agreement between both methods demonstrated that NIR could replace safely XRPD method and vice versa allowing the analyst to choose the method taking into account the advantages and drawbacks of each one.

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