Published in: Journal of Pharmaceutical and Biomedical Analysis (2021), vol. 196, n° March, 113922
DOI: 10.1016/j.jpba.2021.113922
Status : Postprint (Author's version)

Classification of polymorphic forms of fluconazole in pharmaceuticals by FT-IR and FT-NIR spectroscopy

Mohammed Alaoui Mansouri[a,b,1]*, Eric Ziemons[a], Pierre-Yves Sacré[a], Mourad Kharbach[b,c], Issam Barra[b,d], Yahia Cherrah[b], Philippe Hubert[a], Roland Djang'eing'a Marini[a], Abdelaziz Bouklouze[b]

[a] *University of Liege (ULiege), CIRM, Vibra-Santé HUB, Laboratory of Pharmaceutical Analytical Chemistry, CHU, B36, B-4000, Liege, Belgium*

[b] *Bio-Pharmaceutical and Toxicological Analysis Research Team, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, University Mohammed V, Rabat, Morocco*

[c] *Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, CePhaR, Vrije Universiteit Brussel (VUB), Laarbeeklaan 103, B-1090, Brussels, Belgium*

[d] *Center of Excellence in Soil and Fertilizer Research in Africa, Mohammed VI Polytechnic University, Benguerir, Morocco*

KEYWORDS: Polymorphic forms; Fluconazole; FT-IR spectroscopy; FT-NIR spectroscopy; PLS-DA; Classification

ABSTRACT

The main goal of this work was to test the ability of vibrational spectroscopy techniques to differentiate between different polymorphic forms of fluconazole in pharmaceutical products. These are mostly manufactured with fluconazole as polymorphic form II and form III. These crystalline forms may undergo polymorphic transition during the manufacturing process or storage conditions.

[1] **Corresponding author at: University of Liege (ULiege), CIRM, Vibra-Santé HUB, Laboratory of Pharmaceutical Analytical Chemistry, CHU, B36, B-4000, Liege, Belgium*.

Therefore, it is important to have a method to monitor these changes to ensure the stability and efficacy of the drug.

Each of FT-IR or FT-NIR spectra were associated to partial least squares-discriminant analysis (PLSDA) for building classification models to distinguish between form II, form III and monohydrate form. The results has shown that combining either FT-IR or FT-NIR to PLS-DA has a high efficiency to classify various fluconazole polymorphs, with a high sensitivity and specificity. Finally, the selectivity of the PLSDA models was tested by analyzing separately each of three following samples by FT-IR and FT-NIR: lactose monohydrate, which is an excipient mostly used for manufacturing fluconazole pharmaceutical products, itraconazole and miconazole. These two last compounds mimic potential contaminants and belong to the same class as fluconazole. Based on the plots of Hotelling's T^2 vs Q residuals, pure compounds of miconazole and itraconazole, that were analyzed separately, were significantly considered outliers and rejected. Furthermore, binary mixtures consist of fluconazole form-II and monohydrate form with different ratios were used to test the suitability of each technique FT-IR and FT-NIR with PLS-DA to detect minimum contaminant or polymorphic conversion from a polymorphic form to another using also the plots of Hotelling's T^2 vs Q residuals.

1. Introduction

Polymorphism is a characteristic where a drug substance can present one or more crystalline form due to different molecule arrangements; thus can include different solid varieties of crystalline forms. Sometimes, the crystal form is known by solvate when it has amount of solvent and it is known as hydrate if the solvent is water [1–4].

Each polymorphic form has different physico-chemical properties [5]. Variations at the level of physico-chemical properties could have an impact on dissolution rate and bioavailability, hence, the therapeutic effect of the drug substance might be influenced [6]. The manufacturing process and the storage conditions are considered as the main factors that have an impact on polymorphic transformation [7,8]. Thus, looking for a reliable analytical technique to control and monitor polymorphs of drug substance in drug products is mandatory to ensure the quality of pharmaceutical products.

The analysis of polymorphic forms of drug substance has been carried out by both destructive and non-destructive techniques. Destructive techniques are represented in differential scanning calorimetry (DSC). The main challenge of this technique is the interconversion of polymorphic forms

of the drug substance that could be occurred during the analysis [9]. Non-destructive techniques are summarized in X-ray powder diffraction (XRPD) and vibrational spectroscopic techniques [10,11]. The principal advantage of these techniques is that they often do not need any sample preparation. Hence, they are fast in analyzing and acquiring results [12]. Nevertheless, these last cited techniques have some limitations. The main limitation of XRPD is that the morphology of the particle may impact the accuracy of quantitative analysis using XRPD [13]. The main challenge of vibrational spectroscopy is identifying and discriminating between polymorphic forms directly especially in the presence of the matrix that may hamper the identification of fingerprints related to the identity of the polymorphism [14,15]. Thus, associating spectroscopic techniques with chemometric tools is important to uncover more details about polymorphism.

Chemometric tools have already proven their usefulness to discriminate and quantify polymorphic forms such as PCA and PLS and reduce systematic variations by using preprocessing techniques such as standard normal variate (SNV) or multiplicative scatter corrections (MSC) [16]. For example, polymorphic forms were quantified accurately by coupling either of FT-NIR Raman or FT-IR to Partial Least squares regression (PLS-R) [17]. Another example, is applying PCA to FT-IR data that allows detecting which of the four polymorphic forms of cimetidine was present in a pharmaceutical product [18]. In addition to PCA and PLS, another chemometric tool called multivariate curve resolution (MCR) can detect how many polymorphic forms exist in a mixture and identify their pure spectra. This has been combined to FT-IR for following the polymorphic interconversions of cimetidine [19]. Besides that, PLS-DA has been used successfully in food and other applications [20,21]. These applications of chemometric techniques nicely illustrate their efficiency to extract relevant information from the raw spectral data.

Fluconazole, 2-(2,4-difluorophenyl)-1,3-bis(1-H-1,2,4-triazol1-yl)propan-2-ol, is an antifungal triazole. Fluconazole is used to treat superficial Candida infections. It is used for acute therapy of disseminated Candida, for systemic therapy of blast mycosis and histoplasmosis, for dermatophytic fungal infections, and for prophylaxis in neutropenic patients [22–24]. According to recent studies [25,26], fluconazole displays three main polymorphic forms: form I, II and III as well as a monohydrate form. The most stable polymorph is form III. This form is a convert form from the metastable form II, that may convert to the monohydrate form during the storage or compression under specific conditions of humidity and temperature. At the moment, the most marketed forms by Moroccan

pharmaceutical industries are form II and form III while polymorphic form I is considered as unstable based on the recent study [27].

The main objective of this work was to evaluate the qualitative abilities of each vibrational techniques of FT-IR and FT-NIR to classify polymorphic forms of fluconazole in pharmaceutical products as well as investigate the suitability of both vibrational techniques to detect whether there exist any polymorphic conversion or falsified pharmaceutical product of fluconazole

2. Material and methods

2.1. Instrumentation

The FT-IR spectra were acquired in the reflectance mode in the spectral region of 4000−650 cm-1, with an average of 32 scans at resolution of 4 cm-1, using a Frontier FT-IR spectrometer (Perkin Elmer, Waltham, USA) equipped with a diamond crystal ATR device. For each measurement, a fraction of sample is placed onto the diamond surface. the diamond surface was cleaned with acetone and dried between each analysis. The cleaning of the diamond surface was checked spectrally.

The NIR spectra were obtained using FT-NIR spectrophotometer MPA Multi-Purpose FT-NIR Analyzer (Bruker Optics, Ettlingen, Germany) in diffuse reflectance mode in the spectral region of 12500−4000 cm-1 at resolution of 8 cm-1. The average of 32 scans was acquired for each sample by placing the optical fiber probe on the bottom of the glass vial that contains the sample.

2.2. Sample preparation

The main samples that were acquired to build a dataset were:

- Fluconazole polymorphic form II (TCI- Chemicals, Belgium), fluconazole polymorphic form III (Sigma-Aldrich, Belgium), miconazole (Sigma-Aldrich, Belgium), itraconazole (SigmaAldrich, Belgium) and lactose monohydrate (Sigma-Aldrich, Belgium). Fluconazole monohydrate form was obtained based on a reported recrystallization technique [28]. This recrystallization method was carried out by dissolving the fluconazole form- II in milli-Q water under constant stirring at 40 ◦C. The saturated solution was filtered to remove all nuclei, and the filtered solution was cooled in a refrigerator at 5 ◦C. The resulting crystals were rapidly surface dried only, then the polymorphic form of fluconazole monohydrate was checked with FT-IR and the obtained spectrum was

compared to the spectrum of previous studies [26]. The pure polymorphic forms were gently mixed using pestle and mortar and transferred into vials for analysis.

- Commercial pharmaceutical products (50 mg of fluconazole) were acquired at a local drugstore. The average weight of each capsule content was 150 mg. These commercial products consist of two groups:
- The first group is composed of 14 capsules containing polymorphic form II of fluconazole.
- The second group is composed of 17 capsules containing polymorphic form III of fluconazole
- Eleven samples of binary mixtures were prepared. These consisted of 50 mg of fluconazole containing: 1, 2.5, 5, 10, 20, 50 80, 90, 97.5 and 99 % (w/w) of polymorphic form II of fluconazole with the remaining of mass balance of monohydrate form of fluconazole. These binary mixtures were mixed gently using pestle and mortar in order to ensure their homogeneity prior to the transfer to vials for FT-IR and FT-NIR analysis.

2.3.Multivariate data analysis

2.3.1.DATASETS

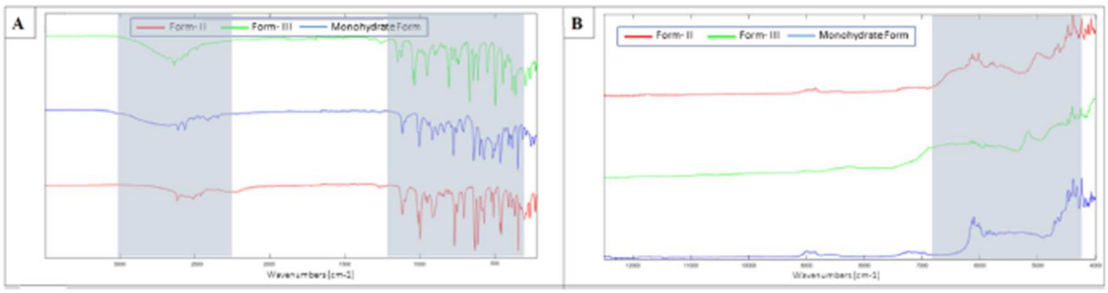
PLS-DA models were developed based on the partial least square algorithm – discriminant analysis using the PLS Toolbox V8.2.1 (Eigenvector research INC, USA) running on Matlab (R2018b) (The Mathworks, USA).

The dataset is composed of three main parts: training, test and suitability set. The training set was used to develop PLS-DA models for three polymorphic forms of fluconazole while test and suitability set were used to test developed models.

A data set was splitted using Kennard-Stone algorithm to provide uniform coverage into Training and test set consisting of:

- samples spectra obtained from mixing 50 mg of each pure polymorphic form of fluconazole with 100 mg of lactose monohydrate in order to be closed from the drug formulation.
- samples from capsules of pharmaceutical products.

Figure 1. Spectra of Fluconazole. Pure polymorphic forms of (A): FT-IR; (B): FT-NIR



A Suitability set was used to test how the built PLS-DA models for FT-NIR and FT-IR can detect minor polymorphic transformations and prove its suitability for samples that do not contain fluconazole.

Here, suitability set is composed of:

- Eleven different spectra of binary mixture of polymorphic form-II and monohydrate form from 1% to 99 % (w/w)
- Three spectra of itraconazole, miconazole and monohydrate lactose were included.

2.3.2. FT-IR AND FT-NIR PREPROCESSING

Before developing the PLS-DA models, the spectra need to be preprocessed to improve discrimination results. We used first derivative as preprocessing method followed by mean centering for FT-IR spectra and we applied standard normal variate (SNV) with mean centering for NIR spectra.

Partial least squares - discriminant analysis (PLS-DA) model were developed based on the training sets and also built only on a range of wavelengths containing the significant variables that are considered as fingerprints of each polymorphic form.

PLS-DA is a supervised classification method that depends on X (FT-NIR or FT-IR spectra of fluconazole polymorphic forms samples) and Y (classes of different polymorphic form) matrices to develop a discriminative calibration model. It is based on reducing the data to scores and loading matrix which permit looking for the most optimal latent variables by maximizing the covariance between X and Y. In case of PLS-DA, Y contains a qualitative variable that identifies different classes of polymorphic forms of fluconazole. Therefore value “1” is attributed to the target class that has to be discriminated from the other alternative classes that have the value of “0”.

Latent variables are chosen based on the lowest error of an appropriate cross validation. This built model is then evaluated by Root Mean Square Error of Calibration (RMSEC) and CrossValidation (RMSECV) from Training set and Root Mean Square Error of Prediction (RMSEP) from the test set used to validate the developed model [29]. To guarantee the reliability of the model in the classification of different classes, a confusion matrix of classification parameters is used to evaluate the performance of PLS-DA models with sensitivity and specificity. The sensitivity is defined as the proportion of the samples of the class that are correctly attributed to the target class (true positives) while the specificity is known as the proportion of the samples that do not belong to the target class to be classified to the alternative class (true negatives) [30].

There are two PLS-DA approaches: the first approach is based on building PLS-DA model for each target class while the other classes are considered alternatives (so called one vs rest classification). This approach would lead us to build three PLS-DA models because of three polymorphic forms of fluconazole. The second approach is PLS-2 regression and it is known as one vs one multiclass PLS-DA model, and it leans on building one model for all calibrated classes.

2.3.3. SUITABILITY SET

Suitability sets were also used to test the developed PLS-DA models. However, in this part we relied on Hotelling's T^2 and Q residual parameters. These parameters helped to elucidate the behavior of the calibrated PLS-DA models towards the new integrated samples. These two parameters (Hotelling's T^2 and Q residual) were used in a plot with a threshold of 0.95 to check the homogeneity of the dataset and detect if there exist any outliers. The Q statistic is used to check how well each sample conforms to the model. The Q value is measured by the difference between the original data and the data reconstructed based on the calibrated model. They are associated to each sample of the dataset and large Q values indicate samples that have large out-of-model residuals. Hotelling's T^2 statistic represents the variation in each sample within the model; it is a measure of the sample distance from the center of the model. A sample with a large Hotelling's T^2 value means that this sample has an influence on the developed model.

3. Results and discussion

3.1. FT-IR and FT-NIR spectra of fluconazole polymorphic forms

Fig. 1 reports different recorded spectra for the three pure polymorphic forms of fluconazole. The spectra agree with previously reported spectra of FT-IR and NIR that have been already analyzed and confirmed by XRPD [25,26]. Improving the discrimination ability of the PLS-DA models has been focused on the spectral regions that are responsible for the polymorphic forms. In this case, building PLS-DA models were limited to the region of 3500−2800 cm-1 and 1670−760 cm-1 for FT-IR and from 9000 to 4500 cm-1 for FT-NIR.

3.2. Exploring datasets

Before developing the PLS-DA models, the homogeneity of pharmaceutical products belonging to the same polymorphic form needs to be checked to ensure that there is not any difference between batches of the same polymorphic forms. This is why PCA was used in order to verify their homogeneity. Based on the PCA plot in Fig. 2 using three components for both FT-NIR or FT-IR data, it is noticed that pharmaceutical products that belong to polymorphic form II or the two pharmaceutical products that are from form III are homogenous. It can be summarized that no tablet or pharmaceutical product that may have a polymorphic transformation.

Another step to verify the homogeneity of the entire datasets is done based on the parameters of Hotelling's T^2 and Q residuals. According to these plotted parameters in Fig. 3, it is observed that most samples have a low Hotelling's T^2 and Q residual values. Nevertheless, some samples are located above the threshold. These samples were tested by removing them and comparing RMSECV and RMSEP before and after removing these samples. Since there are no changes in the values of RMSECV and RMSEP, and thus no sample behave as an outlier whether in FT-IR or FT-NIR spectra.

Figure 2. *PCA for the data homogeneity; (A): FT- IR; (B): FT- NIR; Form- III (green square); Form-II (red diamond); Monohydrate form (blue triangle).*

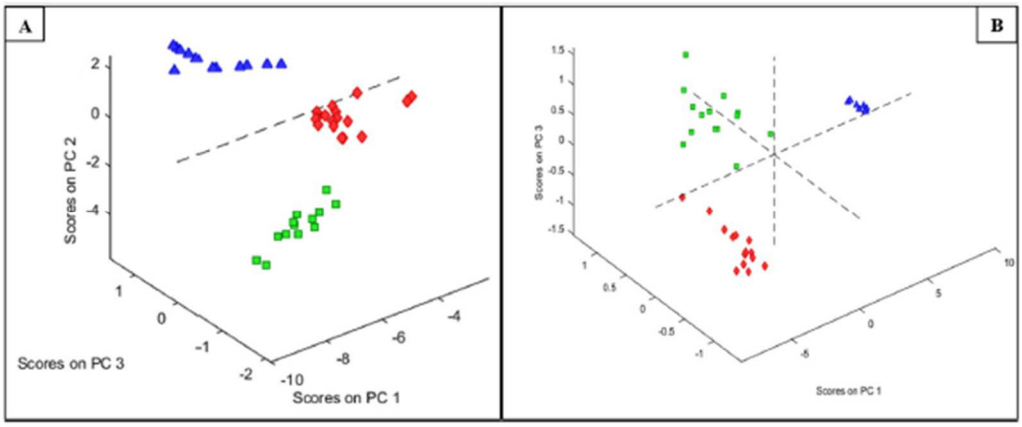
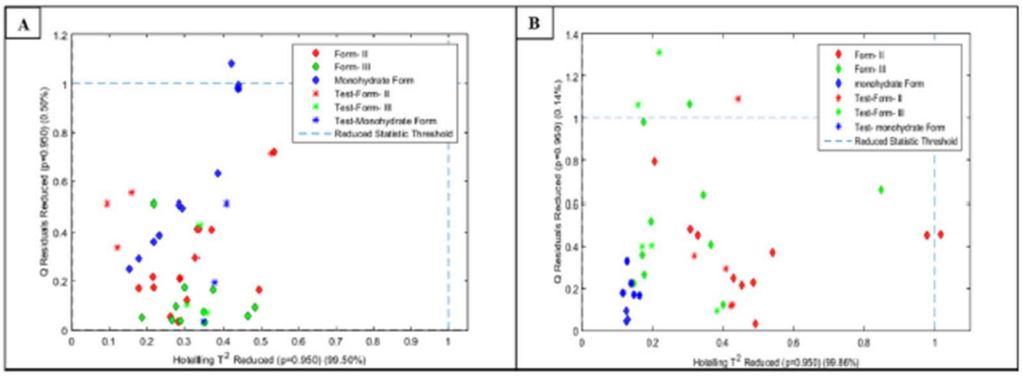


Figure 3*. Hotelling T^2 VS Q Residuals of the training and validation set. (A): FT-IR; (B): FT-NIR;*



3.3.Development of PLS-DA models for FT-IR and FT-NIR spectra

A PLS-DA model was built for each instrument based on the training set. The optimal number of latent variables was chosen using a cross-validation of venetian blinds with 6 splits. We found that five latent variables for FT-NIR and three for FT-IR minimized the RMSECV.

The parameters of the PLS-DA models are shown in Table 1. These results are examined using accuracy that represents the RMSEP, specificity and selectivity. Based on the obtained results, it is noticed that there is a concordance between RMSECV and RMSEP indicating the accuracy of the PLS-DA model of FT-IR and FT-NIR. These results were confirmed by looking to the discriminant plots of the three polymorphic forms of fluconazole in Fig. 4. The PLS-DA models provides 100 % of specificity and selectivity for both FT-IR and FT-NIR datasets.

3.4. The suitability test of PLS- DA models

The suitability test is included in this work to investigate the robustness of the developed PLS-DA models with new samples that are different from samples that have each of the three polymorphic forms and used calibration set. Two kinds of samples were integrated: first category includes samples that do not have any of the three polymorphic forms. In this case, we included the sample lactose monohydrate as the excipient most commonly used in commercial fluconazole drug products. Each of Miconazole and itraconazole were also included since they belong to the same class of fluconazole. The second set contains fluconazole in a binary mixture: 1) fluconazole form- II, which is the metastable form and may convert to fluconazole monohydrate, and 2) the monohydrate form in different ratios from 1 to 99 %. Hotelling's T^2 and Q residuals were used to ensure that these samples are considered to be outliers.

Fig. 5 illustrates how the three samples including itraconazole, miconazole and monohydrate lactose are different from the training set of FT-IR and FT-NIR because they have high values of Hotelling's T^2 and Q residuals versus the set containing the three polymorphic forms. For FT-IR, three samples: miconazole, itraconazole and lactose monohydrate are suspected to be different from the training set due to their high Q residual. In FT-NIR, the three samples were considered outliers because of their high Hotteling's T^2 and Q residual values. Thus, the plot of Hotelling's T^2 and Q residuals values could distinguish these three samples from the three polymorphic forms of fluconazole.

Fig. 6 shows the Q residual vs Hotelling's T^2 of eleven binary mixtures of fluconazole polymorphic forms.

Fig. 6A demonstrates how FT-IR combined to PLS-DA is able to reject different binary mixtures. Based on this plot, it is noticed that binary samples from the ratio of 5–95% can be distinguished since they have values of Q residual and Hotelling's T^2 significantly higher than training set. On the contrary, binary mixture ratios of 1 and 2.5 % were considered belonging to the training set because of their low values of Q residual and Hotelling's T^2 that are similar to the values of training set of polymorphic forms.

Table 1 *- Classification parameters for PLS-DA.*

Spectroscopy technique	FT-IR			FT-NIR		
Polymorphic Forms	Form- II	Form- III	Monohydrate	Form- II	Form- III	monohydrate
Preprocessing	SG1D (2,15) + MC			SNV + MC		
Spectral range	3500–2800 cm^{-1} & 1670–760 cm^{-1}			9000 to 4500 cm^{-1}		
Cross-validation	Venetian blinds			Venetian blinds		
LV	3			5		
RMSEC (%)	11.1	6.3	10.8	4.7	3.4	2.6
RMSECV (%)	12.5	7.2	11.7	4.8	3.5	3.1
RMSEP (%)	14.1	8.1	11.9	6.5	5.5	2.3
Selectivity Calibration (%)	1	1	1	1	1	1
Specificity Calibration (%)	1	1	1	1	1	1
Selectivity Prediction (%)	1	1	1	1	1	1
Specificity Prediction (%)	1	1	1	1	1	1
Discriminant Threshold	0.4	0.3	0.4	0.6	0.3	0.3

Figure 4*. Discriminant plots of three polymorphic forms: (A): FT-IR (the left); (B): FT-NIR (the right).*

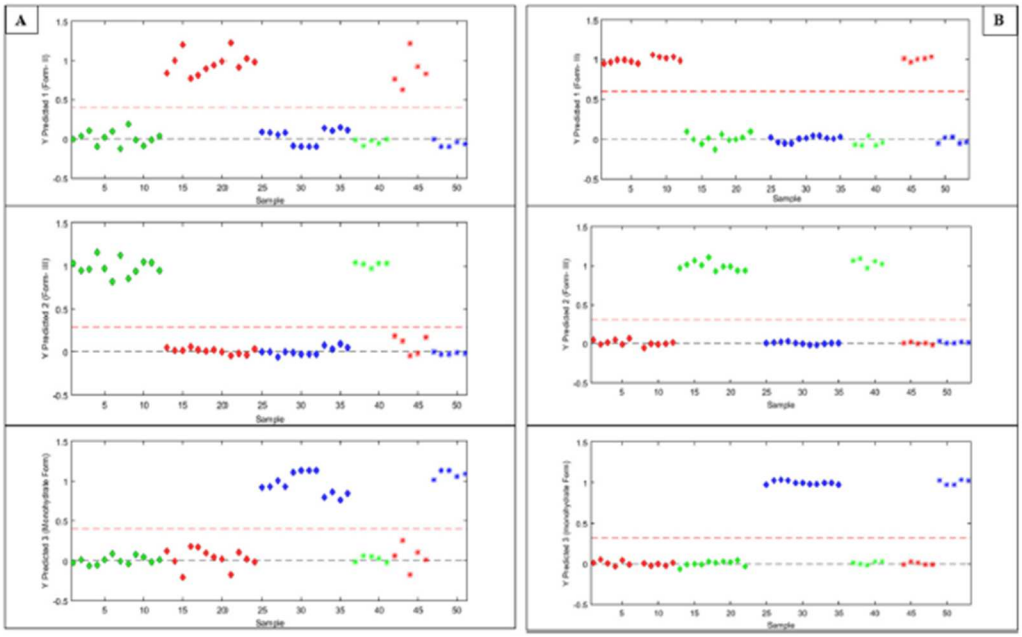


Figure 5*. Hotelling's T^2 Vs Q residual plot of miconazole, itraconazole and monohydrate lactose. A: FT-IR; B: FT-NIR.*

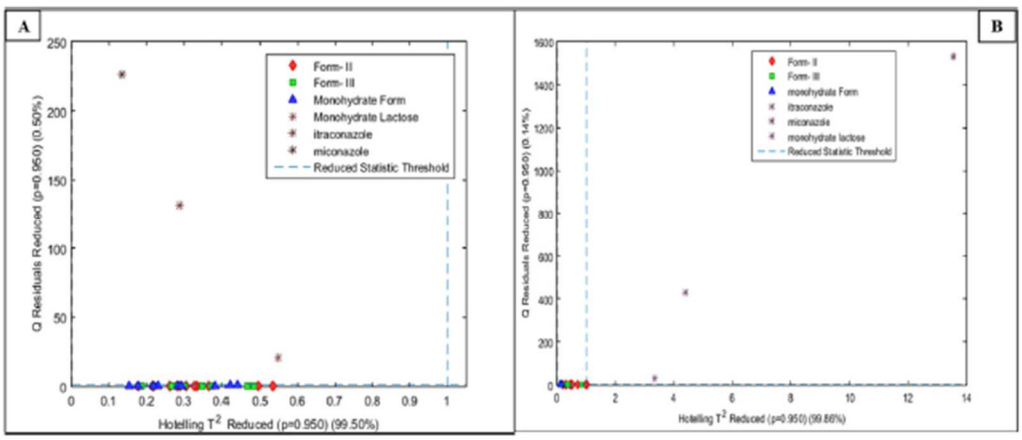


Figure 6. *Hotelling's T^2 Vs Q residual plot of binary mixtures. A: FT-IR; B: FT-NIR.*

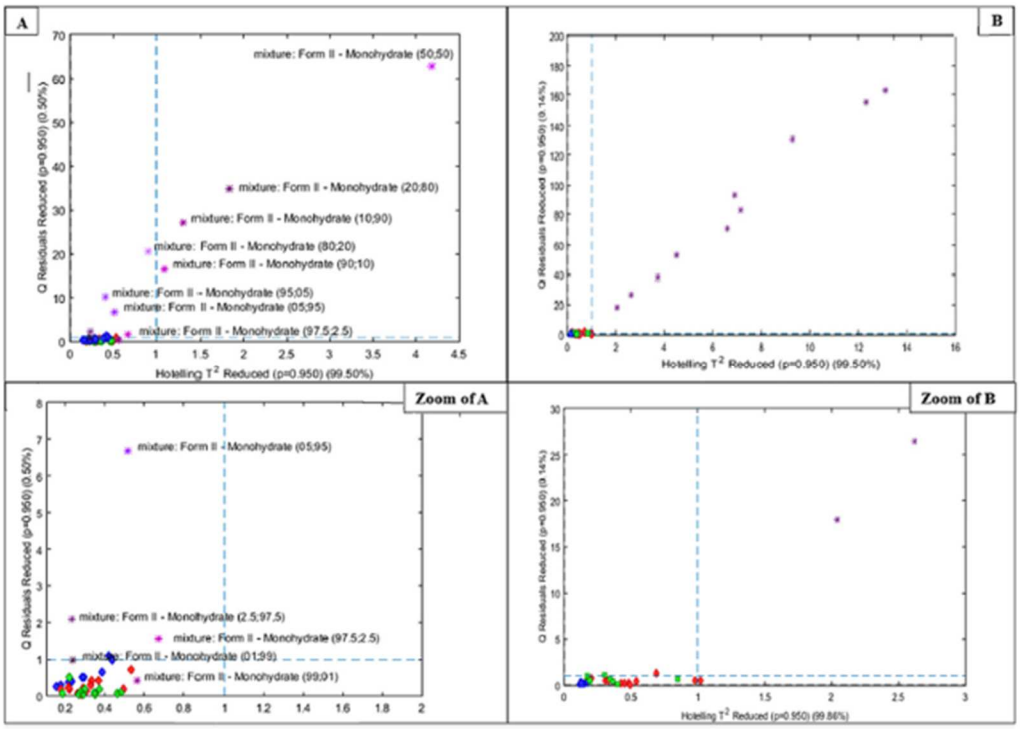


Fig. 6B shows how FT-NIR with PLS-DA can be useful to distinguish different binary mixtures from training set. Based on this plot, all binary mixtures consisted of: form II and monohydrate form are well identified as outliers versus the three calibrated polymorphic forms.

Based on these figures, it can be concluded that FT-NIR is able to detect minimum contaminant or conversion of form-II to monohydrate form because of the sensitive property of FT-NIR to hydrates

4. Conclusion

The results obtained with the PLS-DA models proved the suitability and the efficiency of combining vibrational spectroscopy whether it is FT-IR or FT-NIR with chemometric tools for identification and discrimination of fluconazole polymorphic forms. These results were performed based on several parameters summarized in: RMSEP, RMSECV, specificity and sensitivity that proved the ability of associating of FT-IR or FT-NIR to PLS-DA to discriminate between different polymorphic forms in pharmaceuticals. Finally, the suitability of the models was proven by analyzing itraconazole

and miconazole as well as different binary mixtures (form II and monohydrate form) using Hotelling's T^2 vs Q residual plot. It has been confirmed that each of itraconazole, miconazole and lactose monohydrate are different since they have both Hotelling's T^2 and Q residual values significantly that are higher than the three main polymorphic forms whether using FT-IR or FT-NIR. Nevertheless, FT-NIR shows to be more suitable than FT-IR for detecting minor contamination between monohydrate form and form-II due to its high sensitivity to hydrates.

CRediT authorship contribution statement

Mohammed Alaoui Mansouri: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing original draft, Visualization. **Eric Ziemons**: Conceptualization, Validation, Resources, Writing - review & editing, Supervision, Visualization. **Pierre-Yves Sacré**: Conceptualization, Validation, Resources, Writing - review & editing, Supervision, Visualization. **Mourad Kharbach**: Data curation, Formal analysis, Writing - review & editing. **Issam Barra**: Writing - review & editing. **Yahia Cherrah**: Supervision, Project administration, Resources. *Philippe Hubert*: Supervision, Project administration, Funding acquisition, Resources. **Roland Djang'eing'a Marini**: Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Abdelaziz Bouklouze**: Conceptualization, Validation, Resources, Writing - review & editing, Supervision, Visualization, Project administration, Funding acquisition.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2021. 113922.

Declaration of Competing Interest

The authors report no declarations of interest.

References

[1] H.G. Brittain, S.R. Byrn, E. Lee, Polymorphism in Pharmaceutical Solids, 2009, http://dx.doi.org/10.1016/S0168-3659(01)00252-8.

[2] J. Bernstein, J.M. Bernstein, Polymorphism in Molecular Crystals, Oxford University Press, 2002.

[3] X.Y. Lawrence, Pharmaceutical quality by design: product and process development, understanding, and control, Pharm. Res. 25 (2008) 781–791.

[4] H.G. Brittain, Theory and principles of polymorphic systems, Polymorph. Pharm. Solids. 192 (2009) 1.

[5] J. Aaltonen, M. Allesø, S. Mirza, V. Koradia, K.C. Gordon, J. Rantanen, Solid form screening–a review, Eur. J. Pharm. Biopharm. 71 (2009) 23–37, http://dx.doi. org/10.1016/j.ejpb.2008.07.014.

[6] D. Singhal, W. Curatolo, Drug polymorphism and dosage form design: a practical perspective, Adv. Drug Deliv. Rev. 56 (2004) 335–347.

[7] H.K. Chan, E. Doelker, Polymorphic transformation of some drugs under compression, Drug Dev. Ind. Pharm. 11 (1985) 315–332.

[8] G.G.Z. Zhang, D. Law, E.A. Schmitt, Y. Qiu, Phase transformation considerations during process development and manufacture of solid oral dosage forms, Adv. Drug Deliv. Rev. 56 (2004) 371–390.

[9] E. Atef, H. Chauhan, D. Prasad, D. Kumari, C. Pidgeon, Quantifying solid-state mixtures of crystalline indomethacin by Raman spectroscopy comparison with thermal analysis, ISRN Chromatogr. 2012 (2012).

[10] R. Suryanarayanan, Determination of the relative amounts of -carbamazepine and -carbamazepine in a mixture by powder X-ray diffractometry, Powder Diffr. 5 (1990) 155–159, http://dx.doi.org/10.1017/ S0885715600015608.

[11] D.E. Bugay, A.W. Newman, W.P. Findlay, Quantitation of cefepime · 2HCl dihydrate in cefepime · 2HCl monohydrate by diffuse reflectance IR and powder X-ray diffraction techniques, J. Pharm. Biomed. Anal. 15 (1996) 49–61, http://dx.doi.org/10.1016/0731-7085(96)01796-7.

[12] A.A. Bunaciu, H.Y. Aboul-Enein, V.D. Hoang, Vibrational spectroscopy used in polymorphic analysis, TrAC Trends Anal. Chem. 69 (2015) 14–22.

[13] S.N.C. Roberts, A.C. Williams, I.M. Grimsey, S.W. Booth, Quantitative analysis of mannitol polymorphs. X-ray powder diffractometry—exploring preferred orientation effects, J. Pharm. Biomed. Anal. 28 (2002) 1149–1159.

[14] A. Heinz, M. Savolainen, T. Rades, C.J. Strachan, Quantifying ternary mixtures of different solid-state forms of indomethacin by Raman and near-infrared spectroscopy, Eur. J. Pharm. Sci. 32 (2007) 182–192.

[15] S. Jabeen, T.J. Dines, S.A. Leharne, B.Z. Chowdhry, Raman and IR spectroscopic studies of fenamates - Conformational differences in polymorphs of flufenamic acid, mefenamic acid and tolfenamic acid, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 96 (2012) 972–985.

[16] Å. Rinnan, Pre-processing in vibrational spectroscopy – when, why and how, Anal. Methods 6 (2014), http://dx.doi.org/10.1039/C3AY42270D.

[17] V. Bhavana, R.B. Chavan, M.K.C. Mannava, A. Nangia, N.R. Shastri, Quantification of niclosamide polymorphic forms – a comparative study by Raman, NIR and MIR using chemometric techniques, Talanta 199 (2019) 679–688, http://dx.doi.org/10.1016/j.talanta.2019.03.027.

[18] N.L. Calvo, T.S. Kaufman, R.M. Maggio, A PCA-based chemometrics-assisted ATR-FTIR approach for the classification of polymorphs of cimetidine: application to physical mixtures and tablets, J. Pharm. Biomed. Anal. 107 (2015) 419–425, http://dx.doi.org/10.1016/j.jpba.2015.01.016.

[19] N.L. Calvo, R.M. Maggio, T.S. Kaufman, A dynamic thermal ATR-FTIR/chemometric approach to the analysis of polymorphic interconversions. Cimetidine as a model drug, J. Pharm. Biomed. Anal. 92 (2014) 90–97, http://dx.doi.org/10.1016/j.jpba.2013.12.036.

[20] Ł. Górski, W. Sordon, F. Ciepiela, W.W. Kubiak, M. Jakubowska, Voltammetric classification of ciders with PLS-DA, Talanta 146 (2016) 231–236, http://dx.doi.org/10.1016/j.talanta.2015.08.027.

[21] V.A.G. da Silva, M. Talhavini, I.C.F. Peixoto, J.J. Zacca, A.O. Maldaner, J.W.B. Braga, Non-destructive identification of different types and brands of blue pen inks in cursive handwriting by visible spectroscopy and PLS-DA for forensic analysis, Microchem. J. 116 (2014) 235–243, http://dx.doi.org/10.1016/j.microc.2014.05.013.

[22] S.R. Byrn, J.R.R. Pfeiffer, G. Stephenson, D.J.W. Grant, W.B. Gleason, Solid-state pharmaceutical chemistry, Chem. Mater. 6 (1994) 1148–1158, http://dx.doi. org/10.1021/cm00044a013.

[23] K. Richardson, K.W. Brammer, M.S. Marriott, P.F. Troke, Activity of UK-49,858, a bis-triazole derivative, against experimental infections with Candida albicans and Trichophyton mentagrophytes, Antimicrob. Agents Chemother. 27 (1985) 832–835, http://dx.doi.org/10.1128/AAC.27.5.832.

[24] C. Koks, P. Meenhorst, M. Hillebrand, A. Bult, J. Beijnen, Pharmacokinetics of fluconazole in saliva and plasma after administration of an oral suspension and capsules, Antimicrob. Agents

Chemother. 40 (1996) 1935–1937 (Accessed November 18, 2016) http://aac.asm.org/cgi/content/long/40/8/1935.

[25] E. Ziémons, H. Bourichi, J. Mantanus, E. Rozet, P. Lebrun, E. Essassi, Y. Cherrah, A. Bouklouze, P. Hubert, 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, J. Pharm. Biomed. Anal. 55 (2011) 1208–1212, http://dx.doi.org/10.1016/j.jpba.2011.02.019.

[26] H. Bourichi, Y. Brik, E. Essassi, A. Bouklouze, Y. Cherrah, Solid-state characterization of fluconazole, STP Pharma Tech. Prat. Reglementations 17 (2007) 49.

[27] H. Bourichi, Y. Brik, P. Hubert, Y. Cherrah, A. Bouklouze, Solid-state characterization and impurities determination of fluconazol generic products marketed in Morocco, J. Pharm. Anal. 2 (2012) 412–421, http://dx.doi.org/10.1016/j.jpha.2012.05.007.

[28] K.A. Alkhamis, M.S. Salem, R.M. Obaidat, Comparison between dehydration and desolvation kinetics of fluconazole monohydrate and fluconazole ethylacetate solvate using three different methods, J. Pharm. Sci. 95 (2006) 859–870, http://dx.doi.org/10.1002/jps.20605.

[29] S. Wold, M. Sjöström, L. Eriksson, PLS-regression: a basic tool of chemometrics, Chemometr. Intell. Lab. Syst. 58 (2001) 109–130, http://dx.doi.org/10.1016/S0169-7439(01)00155-1.

[30] M.C. Ortiz, L. Sarabia, R. García-Rey, M.D.L. de Castro, Sensitivity and specificity of PLS-class modelling for five sensory characteristics of dry-cured ham using visible and near infrared spectroscopy, Anal. Chim. Acta 558 (2006) 125–131, http://dx.doi.org/10.1016/j.aca.2005.11.038.