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ORIGINAL ARTICLE

Solid-state characterization and impurities determination of fluconazol generic products marketed in Morocco

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Abstract In this paper, we report the results of quality control based in physicochemical characterization and impurities determination of three samples of fluconazole drug substances marketed in Morocco. These samples were supplied by different pharmaceuticals companies. The sample A, as the discovered product, was supplied by Pfizer, while samples B and C (generics), were manufactured by two different Indian industries. Solid-state characterization of the three samples was realized with different physicochemical methods as: X-ray powder diffraction, Fourier-transformation infrared spectroscopy, differential scanning calorimetry. High performance liquid chromatography was used to quantify the impurities in the different samples. The results from the physicochemical methods cited above, showed difference in polymorph structure of the three drug substances. Sample A consisted in pure polymorph III, sample B consisted in pure polymorph II, sample C consisted in a mixture of fluconazole Form III, form II and the monohydrate. This result was confirmed by differential scanning calorimetry. Also it was demonstrated that solvents used during the re-crystallization step were among the origins of these differences in the structure form. On the other hand, the result of the stability study under humidity and temperature showed that fluconazole polymorphic transformation could be owed to the no compliance with the conditions of storage. The HPLC analysis of these compounds showed the presence of specific

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impurities for each polymorphic form, and a possible relationship could be exist between impurities and crystalline form of fluconazole.

1. Introduction

The polymorphism of drugs has been an important issue in the pharmaceutical industry. Although identical in chemical composition, polymorphs differ in bioavailability, solubility, dissolution rate, chemical stability, physical stability, melting point, and many other properties. In particular, the variation in solubility between different polymorphs can affect drug efficacy, bioavailability and safety [1–4].

Fluconazole (2,4-difluoro-1′,1′-bis(1H-1,2,4-triazol-1-ylmethyl) benzyl alcohol – $C_{13}H_{12}F_2N_6O$, 306.27 g/mol — is a triazole antifungal agent with high oral efficiency. It was discovered in 1981, by a group of scientists in Pfizer Central Research in Sandwich, Kent. This molecule is well tolerated, with no major adverse reactions; it shows an optimum combination of antifungal activity, pharmacokinetic profile, and water solubility. Fluconazole is used against superficial and systemic candidiasis and in the treatment of cryptococcal infections for patients with the acquired immunodeficiency syndrome [5–10].

This active pharmaceutical ingredient (API) crystallizes in more than one solid form (polymorphs). First, two polymorphic forms of fluconazole were reported by Gu and Jiang using FT-Raman and X-rays diffraction [11]. Dash [12] and Alkhamis [13] reported that three polymorphs (form I, II and III as designed by the authors) and a monohydrate form have been distinguished by pfizer's scientists. Many solvates of fluconazole were reported in literature but without any pharmaceutical interest [11–15].

The present study was conducted by the Moroccan National Laboratory for the Control of Medicinal Products (LNCM) in the context of a series of surveys of the quality of the medicinal products marketed in Morocco with the aim of comparing the quality of initially-discovered products and the corresponding generics.

The first part of this work focused on the physical aspect of the purity issue (polymorphism, crystallinity…). All APIs were analysed by X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). These methods put in evidence the differences between APIs.

The second one was on controlling the impurities in fluconazole API, through high performance liquid chromatography (HPLC), and demonstrating whether there is a relationship between the presence of specific impurities to each APIs and its polymorphic form.

We highlighted the importance of solvents used in the process of synthesis and purification which could be at the origin of these differences and determined the stability of each crystalline form under the effect of temperature and humidity.

2. Experimental

2.1. Chemicals

Sample A was supplied by Pfizer (Ireland). Samples B and C were manufactured in India. All three samples consisted in very fine white powder. The fluconazole contents were 99.9, 99.6 and 100.3%, respectively.

The primary reference standard used was that of the US Pharmacopoeia (Cat. No. 1271700).

Impurities reference standards [IMPI (i), IPMII (h), IMPIII (a), IMPIV (g), and IMPV (f)] were provided by a pharmaceutical laboratory located in India.

The monohydrate was prepared by storing sample A under controlled relative humidity and temperature conditions ($RH > 70\%$; 25 °C). All the samples were delivered in sealed containers at room temperature and the various analyses were conducted under controlled relative humidity and temperature conditions.

2.2. Apparatus

2.2.1. Loss on drying

On drying the losses of the various fluconazole samples were determined as the United State Pharmacopoeia monograph on fluconazole (USP 29).

2.2.2. X-ray diffraction

The X-ray diffractograms of the powders were recorded between 5 and 35° (2θ), at room temperature, using a Philips X'Pert-Pro spectrometer fitted with an ultrafast detector X'Celeratar and a copper anticathode ($\lambda = 1.5404$ Å, 45 kV, 40 mA). The diffractograms were recorded with a step width of 0.017°(2θ) and a count time of 11.16 s per step. The mixtures were prepared by grinding together known quantities of pure polymorphs III and II using agate pestle and mortar.

2.2.3. Fourier-transform infrared spectroscopy (FTIR)

The transmission FTIR spectra were recorded at room temperature using a Bruker-Tensor 27 spectrometer. Each spectrum was an average of 20 scans at 4 cm^{-1} resolution over the range 4000 to 400 cm^{-1}. Transparent, homogeneous disk dilutions were prepared by grinding 1 to 3% of test product with potassium bromide.

2.2.4. Differential scanning calorimetry (DSC)

The DSC thermograms of the fluconazole samples were recorded using differential scanning calorimeter DSC 2920 (TA instruments), from 70 to 145 °C, at a rate of 0.5 °C/min. Test samples of weight 5 to 10 mg were weighed in crimped aluminium sample pans.

2.3. Polymorph transformations

2.3.1. Effect of the crystallization solvents on the crystalline forms

All crystallisations were obtained by dissolving the original crystalline powder form (sample A: form II, sample B: form III, sample C: form II+III+monohydrate) in different solvents under constant stirring. Crystallization of each sample was performed separately in isopropanol, hexane+isopropanol

(50:50, v/v), and methanol+water (50:50, (v/v). The solutions were filtered into a warmed conical flask through filter paper. The evaporation of the filtered solutions was performed under different conditions (20 °C±2 °C and RH% 50±5; 20 °C± 2 °C and under vacuum), respectively during 72 h. The crystals obtained was stored in amber glass bottles and kept in desiccators over silica pellets at room temperature. Small quantities were taken from each sample and analyzed with time by FTIR and/or PXRD.

2.3.2. *Effect of temperature and or humidity on the crystalline forms*

Samples (A, B, C) were stored in the oven at 40 °C, 70 °C and 120 °C, respectively during one month. On the other hand, the effect of the relative humidity on the stability of the crystals was performed by storing the three samples at ambient temperature but at different relative humidity (HR% 50±5 and HR% more than 70%) during different times ($t=1$; $t=2$; $t=7$; $t=15$days and 1; 2; and 3 months. Small quantities were taken from each sample and analyzed with time by FTIR and/or PXRD.

2.4. *Chromatographic analysis*

HPLC analyses were performed on an Agilent series 1100 apparatus, equipped with an UV–visible diode array detector. The detection was carried out at $\lambda=261$ nm, with 1 mL/min throughput.

2.4.1. *Fluconazole identification and dosage*

The column used to identifying and dosing fluconazole is a Nucleosil one ($100C_{18}$, 125 mm × 4 mm, 5 µm). The mobile phase was composed of acetate buffer (pH 5.0±0.05; 0.01 M), methanol and acetonitrile (v/v; 70: 20: 10).

2.4.2. *Fluconazole impurities identification and dosage*

Impurity IMPI (i) dosage method **(Method 1):** the column used is a Nucleosil one (100C18, 125 mm × 4 mm, 5 µm).

The mobile phase is composed of a buffer of ammonium dihydrogen phosphate and hexane sulphonic acid monohydrate salt, adjusted at pH 4.0±0.05 with phosphoric acid, and acetonitrile (v/v; 85:15).

Impurities IMPII (h), IMPIII (a), IMPIV (g) and IMPV (f) dosage method **(Method 2)***:* the column used is a Nova-Pack one ($100C_{18}$, 150 mm × 3.9 mm, 4 µm). Separating these impurities was carried out in a gradient mode, with a mixture of acetate buffer at pH 5.0±0.05; acetonitrile and methanol as described in Table 1.

Table 1 Gradient for separating impurities: IPMII (h), IMPIII (a), IMPIV (g) and IMPV (f).

Gradient	Time (min)	Acetonirile (%)	Buffer (%)	Methanol (%)
Step0	10	5	80	15
Step1	20	55	30	15
Step2	3	55	30	15
Step2	2	5	80	15

3. Results and discussion

3.1. *Loss on drying*

The loss on drying results obtained for the three samples A, B, and C are 0.46%, 0.31%, and 0.75%, respectively. The values of sample A and B were less than the norm (0.5%) which is recommended by the USP monograph on fluconazole (USP 29), while the sample C had a volatile materials content that was greater than required value (0.5%). The USP monograph cited that fluconazole must be stored in a sealed container at a temperature under 30 °C.

3.2. *X-ray diffraction*

Comparison of the diffractograms (Fig. 1A) showed that all the samples generated characteristic diffraction lines between 5 and 35° (2θ). The samples thus consisted in different polymorphs and/or phase mixtures. The diffraction lines of sample

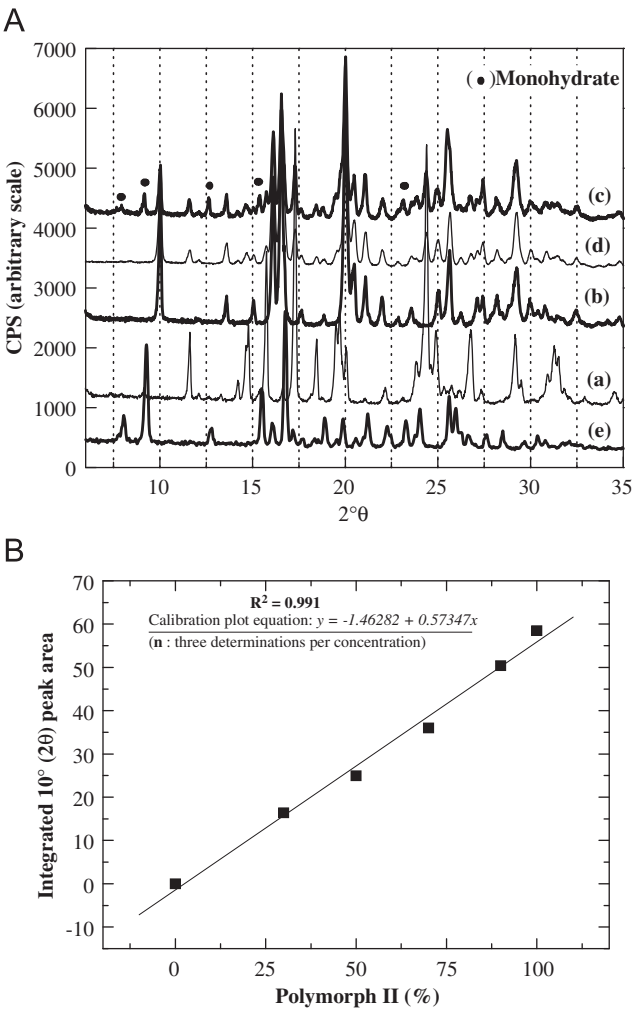


Figure 1 A. X-ray diffractograms: (a) sample A, polymorph III; (b) sample B, polymorph II; (c) sample C; (d) primary reference standard; and (e) monohydrate. B. Calibration plot of the change in integrated 10°(2θ) peak area as a function of polymorph II concentration.

A were identical to those of crystalline polymorph III [11–15], while sample B matched polymorph II [11–15,17].

Neither the primary standard nor sample C matched any previously described polymorph. The primary standard (d) showed the coexistence of diffraction lines associated with polymorphs II and III. In order to further elucidate the composition of sample C, mixtures containing various percentages of pure polymorphs II and III were prepared. Quantitative determination was conducted on the integrated intensity of the peak located at about 10°(2θ), which is specific to form II [17]. Using the calibration plot of the change in the integrated 10°(2θ) peak area as a function of polymorph II concentration (Fig. 2B), the primary standard was found to contain 80% of polymorph II and 20% of polymorph III. The findings reported by Gu et al. also enabled XRD discrimination between crystalline polymorphs II and III [11]. In those authors''s study, determination was conducted on a mixture containing 10% of polymorph II and 90% of polymorph III.

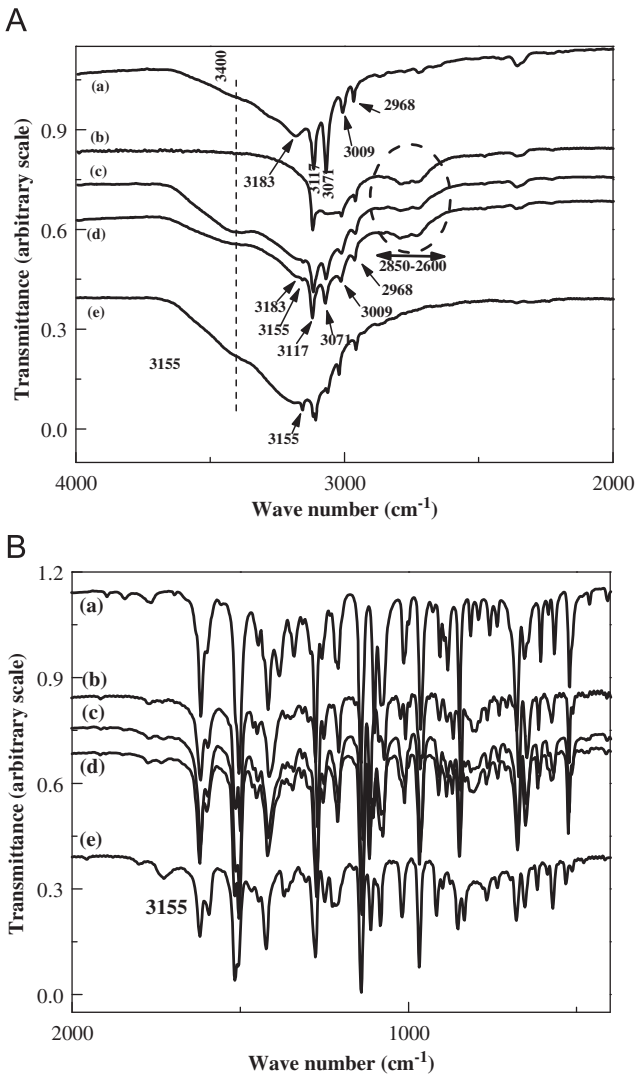


Figure 2 FTIR spectra of the various samples: (a) sample A: polymorph III; (b) sample B: polymorph II; (c) sample C; (d) primary reference standard and (e) monohydrate. A represents the frequency range: 4000–2000 cm^{-1} and B represents range: 2000–400 cm^{-1}.

Alkhamis et al. [13] have reported that polymorph II is a metastable form that is converted to the more stable polymorph III. The diffractogram of the monohydrate form prepared in the present study matched that reported in the literature [12,15] but was not observed in any of the diffractograms recorded on samples A and B. The monohydrate was thus probably not present, only present at levels less than the limit of detection, or present in an amorphous state.

The diffractogram recorded for sample C, is markedly similar to that of the primary standard.

However, other lines present in the monohydrate diffractogram were also present at: 8.1; 9.3; 12.77; 15.54 and 23.28° (2θ) (Fig. 1A). Moreover, the loss on drying results had demonstrated that sample C had a volatile material content that was greater than fixed in the USP 29 monograph (0.75%).

Thus sample C consists in a mixture of forms II and III and the monohydrate. Sample C thus probably consists in:

- either, partial conversions of polymorph II (metastable form) to polymorph III (more stable form), which is converted to the monohydrate under the influence of the storage conditions,
- or a mixture of two batches of API from different sources.

Polymorph I, which can be obtained by synthesis [13] or result from dehydration of the monohydrate [15], was not detected in any of the samples.

3.3. *Fourier-transform infrared spectroscopy*

The samples were also examined using FTIR spectroscopy, one of the methods most widely used to elucidate crystalline polymorphism. As Fig. 2 shows, the spectra recorded on the three samples (A, B and C) and the primary reference standard exhibit noteworthy differences. Samples A and B clearly consisted in polymorphs III and II [12–15,18].

Gu and Jiang have shown that Fourier-transform Raman diffusion spectroscopy enabled complete characterization of a mixture of fluconazole polymorphs III and II [11]. The present study evidenced these differences using transmission FTIR spectroscopy.

The fluconazole functional groups exhibit characteristic bands. After the analysis and comparison of the FTIR spectra recorded on the three samples, we reported in Table 2 a list of the observed wave numbers, with the suggested assignments to vibrations of the various functional groups present in the molecule. The principal structural differences are attributed to the vibrations associated with the triazole group, the 2,4-difluorobenzyl group and the propane backbone.

With regard to the primary reference standard, the frequency range from 4000 to 2000 cm^{-1} is similar to those of polymorphs II and III. The standard spectrum included the bands at 3183, 3117, 3071, 3009 and 2968 cm^{-1} that are characteristic of polymorph III together with those between 2850 and 2600 cm^{-1} characteristic of polymorph II. For the frequency range between 2000 and 400 cm^{-1}, for each of the bands located at 1274, 1210, 1139, 967, 849 and 677 cm^{-1}, a contribution from at least two of the bands characteristic of polymorphs II and III are observed. The bands at 1104 and 609 cm^{-1} characteristic of polymorph III and those at 1116 and 616 cm^{-1} in the spectrum of polymorph II are present.

Table 2 IR absorption bands characteristic of the groups present in the samples.

Assignment bands	Form III (cm^{-1})	Form II (cm^{-1})	Monohydrate (cm^{-1})
Residual water	3400 (sh)		3400 (br-m)
H-bonded OH str	3183 (br—m)		3185 (br-s)
Water			3156 (d-sharp-v-s)
CH str. Triazole ring	3117 (sharp-s)	3121 (sharp-m)	3116–3107 ((d-sharp-v-s)
CH str.Arom. ring	3071 (sharp-s)	3065 (br-m)	3063 (sharp-s)
CH str.Arom. ring	3009 (sharp-w)	3014 (sharp-m)	3020 (sharp-m)
CH2 str	2968 (sharp-w)	2794–2763–2738 triplets (sharp-w)	
Overtones and combs1,2,4	1899–1844–1772 triplet		
trisubst.Arom.ring	(sharp-v-w)	C=C str.of 2,4	
Difluorobenzyl group	1620 (sharp-s)	1620 (sharp-m)	1620 (sharp-m)
	1602 (w)	1600 (d-v-s)	1593–1506(d-sharp-v-s)
Triazole ring str./C=C str			
Arom ring	1506 (sharp-v-v)1518 (sh)	1516–1504 (d-v-s)	1516–1506 (d-sharp-v-s)
CH2 scissor of propane Backbone		1463 (sharp-w)	1465 (sharp-w) 1469 (sh)
CH2 scissor of propane			
Backbone	1449 (w)	1453 (sharp-w)	1445 (sharp-w)
Triazole ring str	1420 (sharp-m)	1413 (sharp-m) 1407 (sh)	1422 (sharp-s)
C=C str.Arom.ring/ ring str.	1387 (sharp-m)	1371 (sharp-w)	
of triazole group	1367 (v-w) 1353 (v-w)	1360 (sharp-w)	
CH bend of propane backbone	353 (sh)		
C=C str. Arom. Ring	343 (sharp-w)	1345 (sharp-v-w)	1349 (w)
Triazole ring str	1317 (v-w)	1317 (w)	1304 (sharp-w)
	1294 (w)	1300 (sharp-w)	1281 (sh)
OH def./CF str. of 2, 4	1278 (sharp-v-s)	1272 (sharp-v-s)	1276 (sharp-s)
difluorobenzyl group			
β-CH Arom. ring/ring str	1260 (sharp-w)	1254 (sharp-w)	1249 (sharp-w)
of triazole group			
β-CH triazole ring	1220–1211 (d-m)	1210 (sharp-m) 1220 (sh)	1227–1218–1210 Triplet (w)
1160 (sharp-v-w)	1162 (sharp-w) 1202 (sh)		
Ring breathing of triazole			
Group	1144 (sharp-v-s)	1138 (sharp-v-s)	1140 (sharp-v-s)
CF str./C-C str. of propane	1104 (sharp-s)	1116 (sharp-s)	112 (sharp-m)
backbone			
C–OH str./CH def. of 2, 4	1084–1078 (d-s)	1090 (sharp-w) 1075 (sharp-m)	1083 (sharp-m)
difluorobenzyl group	1046 (sharp-v-w)	1050 (sharp-v-w) 1040	
(sharp-v-w)			
β-CH Arom. ring/C-(OH) str.	1017 (sharp-m)	1026–1011 (d-sharp-w)	1019 (sharp-m)
of propane backbone			
Ô-CH triazole ring/ring bend of	966 (sharp-s)	968–960 (d-m)	968 (sharp-v-s)
triazole group			
Triazole ring def	909 (sharp-w)	910 (sharp-w)	917 (sharp-m) 942 (sharp-v-w)
	896 (w)		898 (sharp-w)
γ-CH Arom. Ring	885 (sharp-m)	888 (sharp-w) 870 (sharp-w)	
γ-CH triazole ring	850 (sharp-v-s)	846 (sharp-s)	853 (sharp-m) 834 (sharp-m)
γ-CH Arom ring	818–794 (d-sharp-w)	820 (sh) 810–803 (d-w)	813–801–790–780 (v-w) 767
			(sharp-w) 761 (sh)
γ-CH triazole ring CH_2 rock	760–738 (d-sharp-w)	791 (w) 768 (sharp-w) 733 (sharp-w)	747 (v-w) 736 (sharp-w) 726 (sh)
γ-CH Arom. Ring	702 (sh)	711 (sharp-w)	715 (sharp-v-w)
Arom ring def.	681 (sharp-m)	692 (sh) 674 (sharp-m)	696 (sharp-w) 680 (sharp-m)
Triazole ring def	658 (sharp-m) 648 (sh)	651 (sharp-m)	653 (sharp-m)
Arom. ring def	609 (sharp-w)	616 (sharp-w) 605 (sh)	617 (w) 607 (sh)

Sh shoulder. s : strong. m : medium. w : weak. v : very. br : broad. d: doublet.

The monohydrate is indeed present in the spectrum of the primary standard as shown by the bands at 3155 and 834 cm^{-1}. The same remarks can also be formulated for sample C. However, the intensities of the shoulders and bands of the monohydrate form are more marked (Fig. 2A and B).

The broad band centered at 3400 cm^{-1} and generated by the residual water content is more intense for sample C, confirming the results of the loss on drying study (Section 3.1).

3.4. *Differential scanning calorimetry*

The DSC thermograms of samples A, B and C are shown in Fig. 3. For sample A, a single endothermal peak is observed

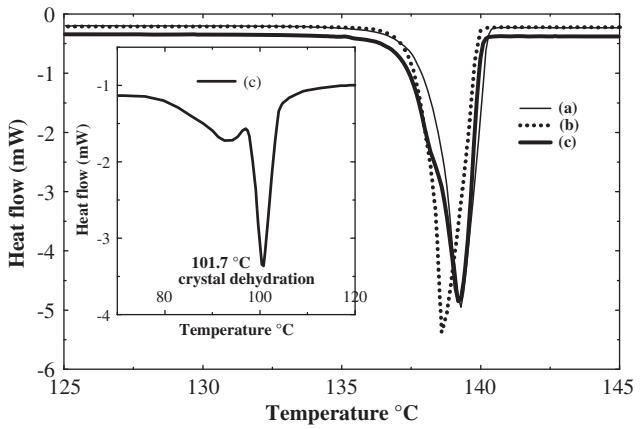


Figure 3 DSC thermograms of the samples: (a) sample A: polymorph III; (b) sample B: polymorph II and (c) sample C.

and is located at 139.3 °C indicating polymorph III [11–14,16]. The melting point of sample B was 138.6 °C indicating the presence of polymorph II [12–14]. The findings confirm the XRD and vibrational spectroscopy results and are concordant with the published data.

However, the sample C thermogram showed the principal endothermal peak of form III at 139.2 °C, with a very pronounced shoulder at about 138.4 °C, indicating the presence of polymorph II. During heating, a quantity of the metastable fluconazole polymorph II was probably converted to the more stable form III.

In addition, an endothermal peak is present at about 101.7 °C, reflecting dehydration of the monohydrate fraction [9]. The thermal profile of sample C further confirms the XRD and FTIR spectroscopic results.

3.5. *Effect of solvent on the crystalline forms*

Crystallization is an operation of purification and crystal formation. The nature of the solvent used is among the gotten essential factors responsible for the final crystalline shape [19–21].

The results of the identification of the different crystalline powders (A, B, C) after their re-crystallization in different solvents followed by evaporation at 20 °C ± 2 °C and RH% 50 ± 5, showed that the three samples were transformed into the monohydrate forms. The form II is found once we realized the crystallization at a temperature of 20 ± 2 °C but under vacuum. These solvents were selected because there were the solvents used in preparation of the APIs as solvents of purification.

However, desolvation, under vacuum at a temperature of 60 °C for 3 h, of the monohydrate form obtained from the form II and form III has allowed us to obtain form III.

During our experiments with different APIs of fluconazole, we found the appearance of the monohydrate in the various samples. This prompted us to conduct a study of stable fluconazole polymorphic forms depending on the temperature and humidity in aim to determine the conditions of conservation of the APIs.

It should be noted that the monograph of European Pharmacopoeia described fluconazole as a hygroscopic powder [22] and the American Pharmacopeia [23] it is mentioned that we must keep this API in brown tight containers at a temperature below 30 °C.

3.6. *Effect of temperature on crystalline transformation*

In order to evaluate thermal transformation, we kept samples: pure form II, pure form III and mixture of forms in oven set at 40 °C, 70 °C and 120 °C during a month. The identification by FT-IR spectroscopy were performed on Small quantities of each sample after 1 days, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 15 days, 20 days, 25 days and last 30 day.

We noted no phase transformation in form II or form III. However, in the literature [14], we found that form II is a metastable form of fluconazole, which is spontaneously transformed into form III at room temperature and under pressure. Indeed, according to this study, the total transformation of form II to III is produced in a period less than a month. The form II that the authors used to demonstrate this transformation was prepared by crystallization of form III in methanol. We have therefore prepared the form II as shown in this study and we have kept it under the same conditions mentioned. No transformation was observed.

On the sample C, after one day we noticed the disappearance of the monohydrate form. This disappearance has been rated for temperatures 40 °C, 70 °C and 120 °C. Indeed, the FTIR spectra recorded showed the coexistence of the single form II and III. Probably, this was the desolvation of the monohydrate form giving rise to the form III (as preshow).

3.7. *Effect of relative humidity on crystalline transformation*

This study was conducted according to the relative humidity, into two distinct conditions: $RH = 50\%$ (± 5) and $RH \geq 70\%$ as was described in Section 2.3.2.

If we consider sample A (form II), we noted the appearance of form II on its FT-IR spectrum after the first day. Probably, part of the form III was transformed into form II under the influence of relative humidity. To our knowledge, this transformation has never been demonstrated in the references dealing with polymorphic transformations of fluconazole. Consequently, we deemed appropriate to analyze this sample PXRD. Indeed, we noted on the XR diffractogram characteristic peak of form II located about $2^{\circ}(2\theta)$.

Regarding the two samples corresponding to form II as obtained from the supplier and that obtained by crystallization of form III into methanol, we have noted that they have the same behavior according to the ambient humidity. Indeed, we noted the formation of the monohydrate form on the 2nd day and then no transformation has been noted for 3 months under the same conditions.

On the sample C (form II+III+monohydrate), we noted no polymorphic transformation at 50 ± 5% (RH) but at (RH) more than 70% it was a total transformation to the monohydrate form.

If we consider the samples kept at ambient temperature and humidity (more than 70%), we noted the same observations as humidity set at 50 ± 5% (RH), except that all samples were transformed to monohydrate form after 3 months.

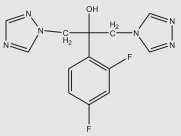
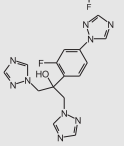
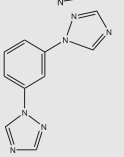
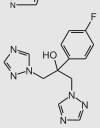
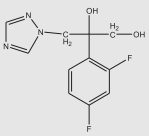
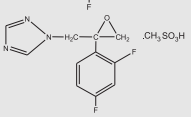
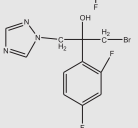
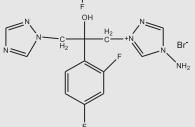
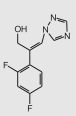
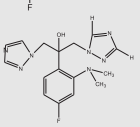
According to the results obtained by the study of stability depending on the temperature and humidity, we can conclude that:

- Crystalline forms II and III of fluconazole were not affected by temperature even at high ones which is in contradiction with references [14].
- The fluconazole APIs are very sensitive to moisture and at relative humidity of $50 \pm 5\%$, we have a transformation of forms III and II in monohydrate form.

3.8. Chromatographic analysis

Before to start the impurities analysis, we have gathered in Table 3, the impurities described by the three API manufacturers (A, B, C) as well as those from the European and US Pharmacopoeias [22,23]. This table equally reports the different impurities structures, their origins, their limit levels as well as their dosage methods. Significant differences appear between manufacturers, whether at the searched impurities

Table 3 Fluconazole impurities.

Origin	Impurities	Structures	Methods
synthesis Impurity	**Impurity III (a)**		CCM
	2-(2,4-difluorophényl)-1-(1H-1-,2,4-triazol-1-yl)-3-(4H-1,2,4-triazol-4-yl)-2-propanol ''mélange racémique''		HPLC
	Impurity b 2-[2-fluoro-4-(1H-1,2,4-triazol-1-yl)-1,3-bis(1H-1,2,4-triazol-1-yl)-2-propanol		CCM HPLC
	Impurity c 1,1′-(1,3-phenylene) di (1H-1,2,4-triazole)		CCM
	Impurity d 2-(4-fluorophényl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol		
Degradation or synthesis product	**Impurity e** 1-[(6RS)-4,6-difluoro-6-(1H-1,2,4-triazol-1yl)cyclohexa-1,4-diényl]éthanone		
	Impurity V (f) 2-(2,4-difluorophényl)-2,3-dihydroxypropyl-1H-1,2,4-triazole		
Degradation product	**Impurity IV (g)** 2-(2,4-difluorophényl)-2,3-epoxypropyl-1H-1,2,4-triazole		**HPLC**
Synthesis impurity	**Impurity II (h)** 2-(2,4-difluorophenyl)-1-bromo-3-(1H-1,2,4-triazol-1-yl)-2-propanol ''fluconazole bromohydrure''		
	Impurity I (i) 2-(2,4-difluorophényl)-1-(1H-1-,2,4-triazol-1-yl)-3-(4H-4-amino-1,2,4-triazonio-1-yl)-2-propanol, bromure ''fluconazole quaternaire''		
	Impurity j [24] Z-2-(2,4-difluorophenyl)-3-(1-H-1,2,4-triazole-1-yl)-2-propen-1-ol		
	Impurity k [24]		
	2-(2-(dimethylamino)-4-fluorophenyl)-1,3-di(3H-1,2,4-triazol-1-yl)propan-2-ol		

a, b, c, d, e, f, g, h and i: impurities cited in European pharmacopoeia [22];.b, c and d: impurities cited in American pharmacopoeia [23].

Table 4 Fluconazole organic impurities dosage results.

Samples and re-crystallisation solvent	Method 1		Method 2				∑Impurities
	IMP I (i) (−4.65 min)	Impurities non identified	IMP_{II} (h) (−23.6 min)	IMP_{III} (a) (−4.1 min)	IMP_{IV} (g) (−18.6 min)	IMP_{V} (f) (−6.7 min)	
Sample A *Isopropanol*	0.38 %	0.01% 0.03 %	ND	0.34 %	ND	ND	0.76 %
Sample B *Isopropanol and Hexane*	0.03 %	0.01 % 0.04 % 0.08 % 0.02 %	ND	ND	ND	0.08 %	0.26 %
Sample C *Méthanol and Eau*	0.35 %	0.03 % 0.15 % 0.04 % 0.02 % 0.02 %	ND	0.38 %	ND	0.08 %	1.07 %

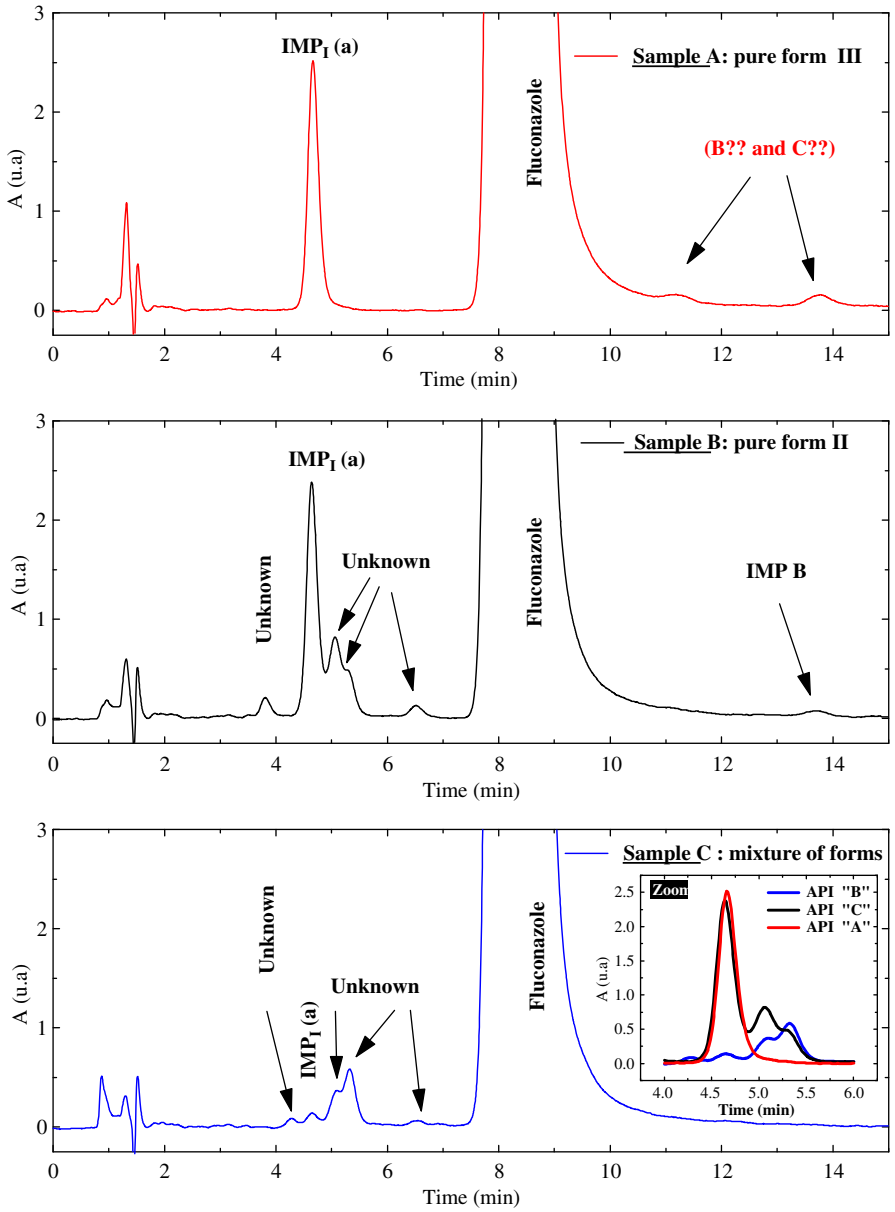


Figure 4 HPLC Chromatograms of fluconazole samples with the IMPI dosage method.

number and nature level, or at the recommended dosage method level. Considering only the three samples belonging to our three suppliers, we observed that the presence of three synthesis impurities (a, b and c) was reported by the manufacturer A, with thin-layer chromatography (TLC) as control method. The same impurities were quantified through HPLC by the manufacturer B. Conversely, the manufacturer C describes only two impurities (a and b) with HPLC as dosage method. The fluconazole European Pharmacopoeia monograph specifies the impurities a, b and c while the US monograph only describes the three impurities b, c and d.

The impurities [IMPI (i), IMPII (h), IMPIII (a), IMPIV (g) and IMPV (f)] coming either from synthesis or degradation and which are mentioned in the European Pharmacopoeia monograph, but are not searched by the three manufacturers, except IMPIII (a).

The results achieved with both dosage methods showed that impurities levels are widely lower than the specifications set, whatever the API origin (Table 4).

In addition, the IMPI dosage method allowed us to drawing the following observations:

- IMPI (TR=4.65 min) is present in the three APIs, at different levels. Actually, as showed in Fig. 4, both samples A and C contain this impurity at a similar level of approximately 0.35%, while sample B contains this impurity in a very low quantity (0.03%);
- Sample A chromatogram contains, in addition to IMPI, two other wide, small surfaced peaks, respectively located at 11.2 and 13.8 min. Both peaks may be due to the a, b or c impurities, since Pfizer, the discovering laboratory, only specifies three impurities, a, b and c;

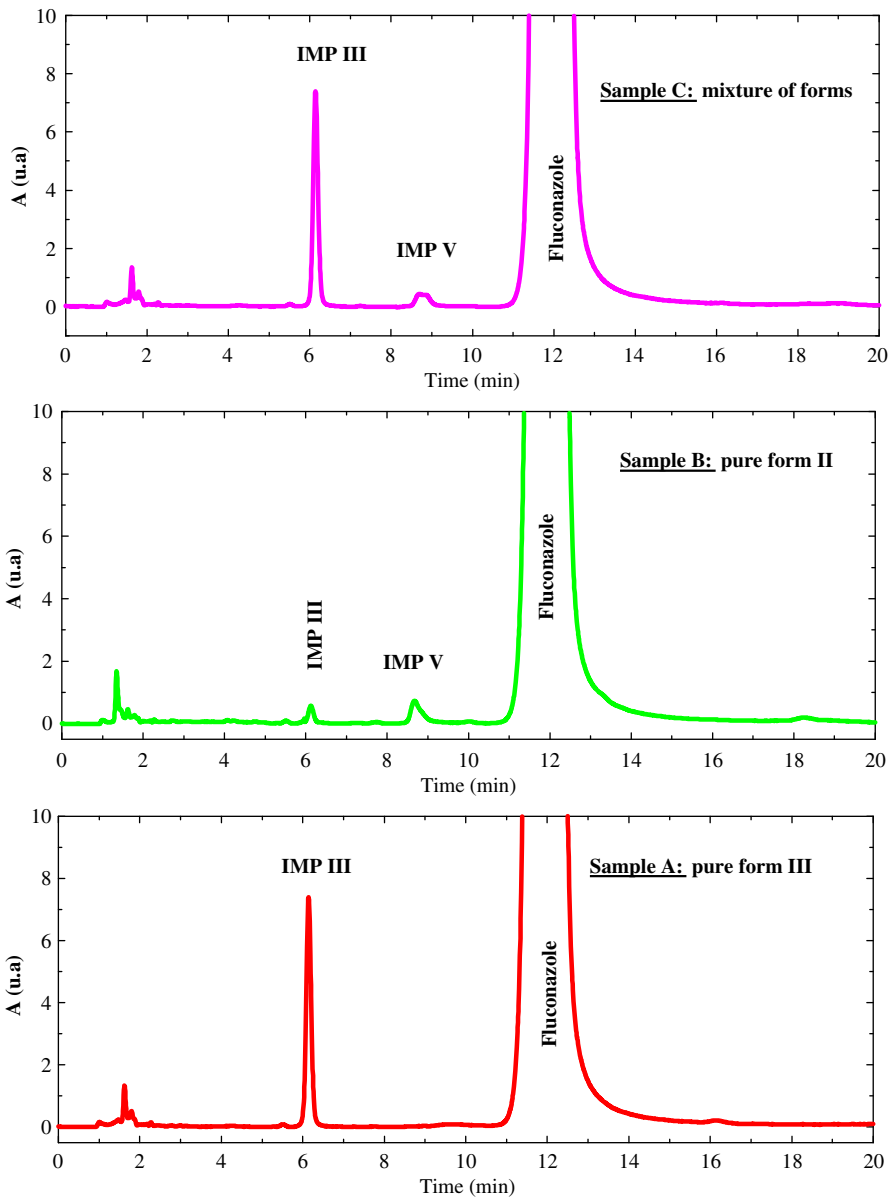


Figure 5 HPLC Chromatograms of Fluconazole samples with IPMII; IMPIII; IMPIV and IMPV dosage related method.

- In addition, we noted, on samples B and C, the appearance of other small surfaced peaks due to unidentified impurities.

If we consider now the second IPMII; IMPIII; IMPIV and IMPV dosage related method, we note that only IMP III is present on sample A, while B solely contains IMPV. Sample C contains both impurities (Table 4).

While examining the results (Fig. 5), we noted that, actually, the sample C related chromatogram is the addition of those related to samples A and B. The same observations were reported, basing on the IMP II, IMP III, IMP IV and IMP V dosage method (Table 4).

Let us remind that sample A is solely composed of the crystalline form III and sample B contains the pure crystalline form II, while C is a mixture of three polymorphic forms: III, II and the monohydrate form.

We also noted differences at APIs manufacturing plans level, in particular at the end purification solvents level, which explains on the one hand, the difference between polymorphic forms and, on the other hand, the difference between the corresponding impurities.

Given the results achieved above, concerning every raw material, the relationship between the different crystalline forms of fluconazole and the possible presence of impurities specific to every polymorph is clearly obvious. It is, therefore, possible, from the impurities determination related chromatograms, to get previous information on the polymorph(s) in which presents the API in question.

4. Conclusion

We noted remarkable differences on the physical and chemical purity of the different APIs. Indeed, physicochemical characterization by XRD, FTIR spectroscopy and DSC enabled differentiation of the three APIs samples. Sample A entirely consisted of polymorph III, sample B entirely consisted of polymorph II, and sample C consisted in a mixture of polymorphs II and III and the monohydrate.

A relationship between fluconazole specific impurities and crystalline form was clarified by using HPLC. It is therefore possible, from the impurities dosage results achieved through HPLC, to distinguish the different fluconazole crystalline polymorphs.

It was interesting to investigate the factors affecting fluconazole polymorphic transformations. In fact, relative humidity proved to transform both polymorphs to monohydrate form. While, no transformations were noted due to the high temperatures, both form II and III showed high stability in high isothermal.

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