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Fluconazole–excipient compatibility studies as the first step in the development of a formulation candidate for biowaiver

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Abstract The biowaiver of bioequivalence studies on class I drugs of the biopharmaceutics classification system (BCS) is aimed mainly at reducing the costs and the exposure of health volunteers to a new pharmaceutical formulation. Fluconazole is an important antifungal agent but in the literature it is not clear whether it belongs to BCS class I or III. Compatibility studies are considered to be the first step in product development and on considering a biowaiver candidate these gain even greater importance since the final product will not be submitted to in vivo tests. The aim of this study was to qualitatively determine the composition of a commercially available fluconazole formulation in the form of capsules with regard to the presence of critical excipients and to carry out compatibility studies by differential scanning calorimetry (DSC). One formulation did not contain sodium lauryl sulfate and contained mannitol, in contrast to the reference formulation, which could hinder the acceptance of the biowaiver. The interaction of fluconazole with microcrystalline cellulose and calcium hydrogen phosphate dihydrate was observed; however, no indication of incompatibility was found in the DSC analysis of the commercial pharmaceutical formulations. These interactions were also studied by Fourier transform infrared spectroscopy, where small changes in the bands were observed, and by X-ray Powder diffraction and scanning electron microscopy that did not evidence any modification in the solid state characteristics.

Keywords Biowaver · Fluconazole · Compatibility studies · Thermal analysis

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Introduction

The biopharmaceutics classification system (BCS) categorizes active pharmaceutical ingredients (APIs) on the basis of their permeability and solubility into four classes class I (high solubility, high permeability), class II (low solubility, high permeability), class III (high solubility, low permeability), and class IV (low solubility, low permeability) [1]. The BCS has gained much attention since its application can reduce the need for clinical studies, serving as a tool to identify compounds eligible for a biowaiver of in vivo bioequivalence (BE) tests. Certain regulatory agencies, for instance, the Food and Drug Administration (FDA), European Medicine Agency (EMA), and World Health Organization (WHO), accept the replacement of BE studies with in vitro assays, thus reducing not only the exposure of healthy volunteers to drug candidates in BE tests but also the costs and time required for the development of pharmaceuticals [2–4].

In 2011, the Brazilian Health Surveillance Agency (ANVISA) published guidelines for the biowaiver of in vivo BE tests for some BCS class I APIs [5]. Lists of APIs candidates for biowaiver have also been published by ANVISA [6], WHO [7], and International Pharmaceutical Federation (FIP) [8].

Fluconazole (Fig. 1) is chemically described as 2,4-difluoro-1′,1′-bis(1*H*-1,2,4-triazol-1-ylmethyl) benzyl alcohol with an empirical formula of $C_{13}H_{12}F_2N_6O$ and molar

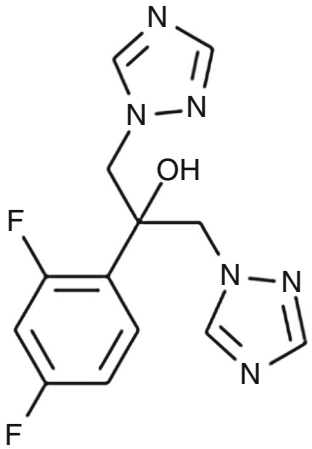

Fig. 1 Chemical structure of fluconazole

mass of 306.3 g mol^{-1} [9, 10]. It is available as tablets for oral administration, as a powder for oral suspension, and as a sterile solution for intravenous use [10, 11]. Instead of tablets, in some countries, including Brazil, fluconazole is available as capsules, usually in a 150 mg dosage.

Fluconazole is a highly selective inhibitor of the fungal cytochrome P450 dependent enzyme lanosterol 14-α-demethylase. This enzyme acts by converting lanosterol to ergosterol. The subsequent loss of normal sterols correlates with the accumulation of 14-α-methyl sterols in fungi and may be responsible for the fungistatic activity of fluconazole. Its spectrum of activity covers a large number of *Candida* spp., but *Candida glabrata* and *Candida krusei* present a dose-dependent susceptibility (*C. glabrata*) or complete resistance [10–13].

Reports in the literature regarding the BCS classification of fluconazole are confusing [2] since it is referred to as either BCS class III [2, 14] or BCS class I [2, 15]. The in-depth characterization and understanding of the physicochemical interactions of an API in the dosage forms is an integral part of pre-formulation studies, during the development of a new pharmaceutical formulation. Although excipients are usually considered to be pharmacologically inert, they can interact with APIs and affect various aspects of the product, such as the organoleptic properties, dissolution, or drug degradation [16–19], impairing its stability and/or bioavailability. The assessment of API–excipient interactions could be considered to be even more critical for medicines which are candidates for the BE biowaiver, since they will not be submitted to in vivo studies.

Formulation scientists have explored diverse thermoanalytical techniques for the early prediction of suitable excipients for the dosage forms to minimize or mitigate the untoward reactions which arise from drug–excipient incompatibility [18]. Differential scanning calorimetry (DSC) represents a leading thermal analysis technique that has been increasingly used for the rapid active screening of incompatibility for pharmaceutical ingredients [20]. When drug–excipient interactions are detected by DSC, the incompatibility should be confirmed using other methods, such as thermogravimetric (TG) analysis, hot stage microscopy, X-ray powder diffraction (XRPD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), solid state nuclear magnetic resonance (NMR) spectroscopy, or high performance liquid chromatography (HPLC) [18].

Fluconazole is only included as a candidate for biowaiver on the Brazilian ANVISA list. Thus, considering that the estimation of drug–excipient interactions is a crucial step in pre-formulation studies during drug development to achieve acceptable stability, bioavailability, and manufacturability of solid dosage forms, and this is considered to be even more critical for medicines which are candidates for the BE biowaiver, the aim of this work was to analyze the composition of commercially available fluconazole capsules and to carry out compatibility studies by DSC, TG, FTIR, XRPD, and SEM.

Materials and methods

Materials

Commercial formulations of fluconazole (reference, generic and similar) were purchased from distinct laboratories within their shelf-life period and were designated as R, for reference fluconazole, G1–G3, for generic formulations and S1–S3 for similar formulations. The fluconazole raw material was kindly donated by EMS S/A (Hortolândia, SP, Brazil). Based on the patient information leaflet, a list of the excipients used in the production of these formulations was compiled (Table 1) and these were then considered in the compatibility studies. The pharmaceutical excipients tested were microcrystalline cellulose PH 101 (Sintética, Capivari, SP, Brazil), magnesium stearate (Henrifarma, São Paulo, SP, Brazil), mannitol (Gemini, Anápolis, GO, Brazil), lactose anhydrous (Sigma-Aldrich, St. Louis, MO, United States), lactose monohydrate (Pharmanostra, Anápolis, GO, Brazil), sodium lauryl sulfate (Viafarma, São Paulo, SP, Brazil), silicon dioxide (Gemini, Anápolis, GO, Brazil), croscarmellose sodium (Adrivan, Diadema, SP, Brazil), starch (Biotec, Pinhais, PR, Brazil), calcium hydrogen phosphate dihydrate (Henrifarma, Cambuci, SP, Brazil), polyvinylpyrrolidone K30 (Biotec, Pinhais, PR, Brazil).

Compatibility studies

The compatibility studies were performed with binary mixtures (1:1; m/m) of fluconazole and each excipient

Table 1 Excipients used in different commercial formulations of fluconazole

Excipient	Fluconazole formulation						
	R	G_1	G_2	G_3	S_1	S_2	S_3
Calcium hydrogen phosphate dihydrate						X	
Croscarmellose sodium		X				X	
Ethyl alcohol				X			
Lactose anhydrous							X
Lactose monohydrate	X	X	X		X		
Magnesium stearate	X	X	X	X	X	X	X
Mannitol				X			
Microcrystalline cellulose				X		X	
Polyvinylpyrrolidone				X		X	X
Silicon dioxide	X	X	X		X	X	X
Sodium lauryl sulfate	X	X	X		X	X	X
Starch	X		X		X		

R reference, *G* generic, *S* similar

present in the commercial capsules. The physical mixtures were vortex mixed for 2 min, and then immediately submitted to the analysis. The reference, generic, and similar formulations were also analyzed.

DSC

The DSC curves were obtained on a Shimadzu DSC-60 cell (Kyoto, Japan) using aluminum crucibles with around 2 mg of samples. The temperature range was from 25 to 300 °C, and the heating rate was 10 °C min^{-1} in a dynamic N_2 atmosphere with a flow rate of 50 mL min^{-1}. The DSC equipment was calibrated with a standard reference of indium (m.p. 156.6 °C; $\Delta H_{fus} = -28.54$ J g^{-1}) and zinc (m.p. 419.5 °C).

Thermogravimetric (TG) analysis

TG experiments were carried out on a Shimadzu thermobalance model TGA-50 (Kyoto, Japan) within a temperature range of 30–600 °C, for the excipients using platinum crucibles with approximately 4 mg of samples, under dynamic N_2 and air atmospheres (50 mL min^{-1}) at a heating rate of 10 °C min^{-1}.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra were obtained for fluconazole, microcrystalline cellulose, calcium hydrogen phosphate dihydrate, and their binary mixtures, on a FTIR Frontier (Perkin Elmer, Massachusetts, USA), within a scan range of 4,000–600 cm^{-1}, with an average of over 32 scans, at a spectral resolution of 4 cm^{-1}. A background spectrum was obtained for each experimental condition.

XRPD

XRPD patterns were obtained on a D2 Phaser diffractometer (Bruker, Massachusetts, USA), with tube of Cu-K_α, in the range of 5–40° (2θ) with a pass time of 1 s and increment of 0.05°. The samples of fluconazole, microcrystalline cellulose, calcium hydrogen phosphate dihydrate, and their binary mixtures were gently placed on the sample holder to avoid preferential orientation problems and the sample holder was kept at 5 rpm during the analyses.

SEM

For acquisition of micrographs a SEM (TM3000, Hitachi, Tokyo, Japan) with backscattered electron detector coupled to an energy dispersive spectrometer (EDS) (SwiftED3000) with silicon detector (SDD of 30 mm^2 and resolution of 161 eV, Cu-K_α) and multichannel analyzer (2,048 channels, 10 eV/channel) was used. The samples were loaded, without any pre-treatment, on aluminum stub fixed on double-sided carbon tape.

Results and discussion

Composition analysis of fluconazole formulations

In most cases, to avoid the risk of bioinequivalence in the development of formulations with BCS class 1 candidates for the BE biowaiver it is advisable to use similar amounts of the same excipients in the compositions of the test and reference products. If a biowaiver is applied to a BCS class III drug substance, the excipients have to be qualitatively the same and quantitatively very similar in order to exclude different effects on membrane transporters. The main restriction relates to the use of the so-called "critical excipients". These excipients (e.g. sorbitol, mannitol, sodium lauryl sulfate, or other surfactants) can affect the bioavailability and should be identified along with their possible impact on the gastrointestinal motility, susceptibility of interactions with the drug substance, drug permeability, and interaction with membrane transporters. Also, the critical excipients should be qualitatively and quantitatively the same in the test product and the reference product for both BCS class I and class III drugs [21–23].

According to Table 1 and on considering the presence of critical excipients, only the G3 formulation does not contain sodium lauryl sulfate and contains mannitol. The G3 formulation also contains ethyl alcohol, suggesting that it

was the only formulation for which wet granulation was employed in the manufacturing process. Since it is a generic formulation, BE studies were carried out and the product was approved. However, being a distinct formulation, it would probably not meet the biowaiver criteria.

DSC compatibility studies

The thermoanalytical characterization of fluconazole is shown in Fig. 2. The DSC curve shows a sharp endothermic event ($T_{peak} = 138.16$ °C; $T_{onset} = 135.70$ °C; ($\Delta H_{fusion} = -114.91$ J g^{-1}), corresponding to the melting point. The TG/DTG curves reveal the fluconazole decomposition in two overlapped steps between 256 and 295 °C ($\Delta m = 99.1$ %). No further events were observed in temperatures above 300 °C.

For the compatibility study, the DSC curves for the pure components are compared to the curves obtained from 1:1 physical mixtures. It is assumed that the thermal properties (melting point, change in enthalpy, etc.) of the blends are the sum of the individual components if the components are compatible with each other. The absence of a peak, a significant shift in the melting peak of the components or the appearance of a new exo/endothermic peak in the physical mixture indicates incompatibility. However, slight changes in the peak shape, height, and width are expected due to possible differences in the mixture geometry [24]. In general, when interaction is observed by DSC it is necessary to investigate the possibility of incompatibility employing other methods.

The thermal profiles of the mixtures fluconazole/croscarmellose sodium, fluconazole/lactose monohydrate, fluconazole/magnesium stearate, fluconazole/polyvinylpyrrolidone, fluconazole/silicon dioxide, fluconazole/sodium lauryl sulfate, and fluconazole/starch can be considered as a superposition of the curves for the fluconazole and the excipients (Fig. 3; Table 2), demonstrating the absence of interaction.

Croscarmellose sodium is commonly used as a disintegrant in tablets and capsules. The DSC curve for fluconazole/croscarmellose sodium (Fig. 3b) shows the characteristic endothermic event of the fluconazole melting point at 133.7 °C and a broad endothermic event in the range of 75–125 °C, corresponding to the loss of adsorbed water. It has been shown by DSC experiments that croscarmellose sodium is compatible with cefpodoxime proxetil [25] and

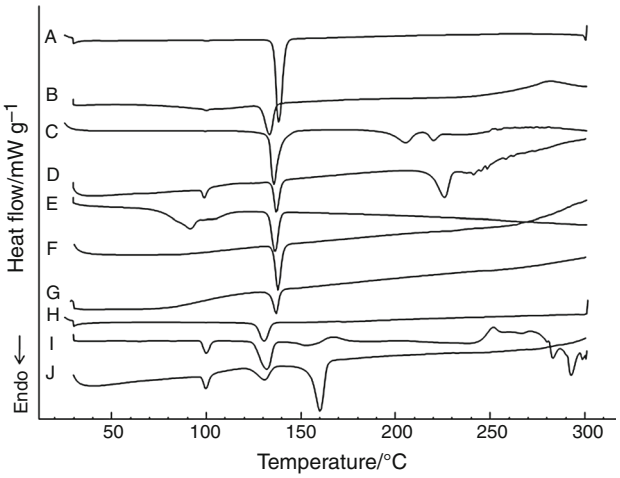

Fig. 3 DSC curves for fluconazole and excipients mixtures (1:1; m/m). *A* pure fluconazole, *B* croscarmellose sodium, *C* lactose monohydrate, *D* lactose anhydrous, *E* magnesium stearate, *F* starch, *G* polyvinylpyrrolidone, *H* silicon dioxide, *I* sodium lauryl sulfate, *J* mannitol

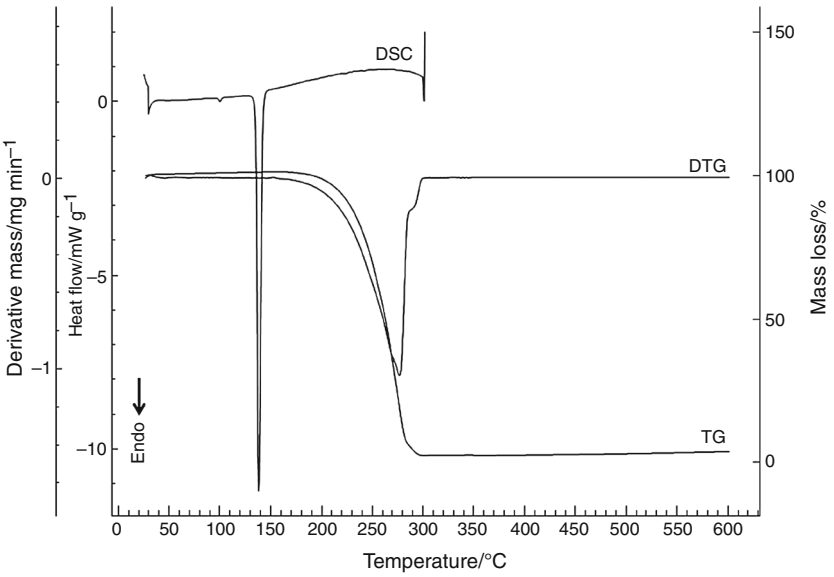

Fig. 2 DSC and TG/DTG curves for pure fluconazole obtained in nitrogen atmosphere (50 mL min^{-1}) at a heating rate of 10 °C min^{-1}

Table 2 Initial temperature (T_{onset}) and peak temperature (T_{peak}) of the melting event and variation in enthalpy (ΔH) of fluconazole and excipients mixtures (1:1; m/m)

	T_{onset}/ °C	T_{peak}/ °C	ΔH fusion/ J g^{-1}
Fluconazole	135.70	138.16	−114.91
FLZ + calcium hydrogen phosphate dehydrate	–	–	–
FLZ + croscarmellose sodium	129.04	133.70	−55.58
FLZ + lactose anhydrous	134.13	136.75	−45.00
FLZ + lactose monohydrate	133.01	135.81	−115.71
FLZ + magnesium stearate	133.08	136.43	−59.78
FLZ + mannitol	123.55	130.78	−41.81
FLZ + microcrystalline cellulose	115.35	125.55	−57.22
FLZ + polyvinylpyrrolidone	93.01	137.12	−40.85
FLZ + silicon dioxide	126.10	130.93	−34.39
FLZ + sodium lauryl sulfate	124.85	132.04	−65.84
FLZ + starch	134.88	137.84	−66.08

metformin [26]. Solid state interactions were observed with sildenafil citrate [27] and enalapril maleate [28].

Lactose serves to dilute or fill tablets and capsules. For the formulations in which lactose monohydrate was identified, this excipient was evaluated. However, when the patient information leaflet mentioned only lactose, anhydrous lactose was used. The curve for fluconazole/lactose monohydrate (Fig. 3c) showed one endothermic peak characteristic of the fluconazole melting point at 135.8 °C, followed by the melting of lactose monohydrate at 205.4 °C [29]. The dehydration of lactose monohydrate was observed as an endothermic event at 141.8 °C when the excipient was analyzed alone (data not shown). This event was probably overlapped with the melting point of fluconazole in the 1:1 mixture, as also can be seen in the higher ΔH fusion value (−115.71 J g^{-1}) for this sample, in comparison to the other binary mixtures (Table 2). The curve for fluconazole/lactose anhydrous (Fig. 3d) demonstrated the melting point of fluconazole at 136.7 °C, without any change in the DSC profile. In contrast, Desai et al. [30] also observed peaks at 86.1 and 136.4 °C in addition to the peak corresponding to the melting of the pure drug at 140.2 °C indicating partial interaction of the drug with lactose. However, the authors did not mention the type of lactose present. Lactose was considered compatible with sibutramine hydrochloride monohydrate [31] and norfloxacin [32] and incompatible with promethazine hydrochloride [33] and acyclovir [34]. Usually, the incompatibilities concerning lactose are due to the Maillard reaction. This reaction is likely to occur between lactose (a reducing disaccharide) and compounds with a primary and/or secondary amine group, usually resulting in brown, or yellow-brown-colored products [29, 34].

Magnesium stearate is used in tablets and capsules as a lubricant. In the fluconazole/magnesium stearate mixture (Fig. 3e) two overlapping endothermic events were observed. The first in the range of 75–101 °C was attributed to the dehydration of magnesium stearate and the second in the range of 96–126 °C was attributed to the melting of the excipient, which is within the reported melting range of 117–150 °C [29]. A third event was observed at 136.43 °C corresponding to the melting of fluconazole. Magnesium stearate was incompatible with nebicapone [35], aceclofenac [36], and acetylsalicylic acid [37].

Starch is a versatile excipient used as a binder, diluent, disintegrant, and thickening agent. The curve for fluconazole/starch (Fig. 3f) showed the endothermic peak for the fluconazole melting point at 137.8 °C. It was incompatible with the antidepressant seproxetine maleate [38] and with the bronchodilator clenbuterol [39]. Starch was compatible with the antihistaminic desloratadine [40] and with the atypical antipsychotic risperidone [41].

Polyvinylpyrrolidone is mainly used as a disintegrant, dissolution enhancer, suspending agent and tablet binder. In tableting, povidone solutions are used as binders in the wet-granulation processes. Similarly to the case of the starch mixture, it was considered that the fluconazole/polyvinylpyrrolidone (Fig. 3g) curve showed the superposition of the individual DSC curves, with the fluconazole melting point at 137.1 °C. Polyvinylpyrrolidone was incompatible with the anti-inflammatory ketoprofen [42], antihypertensive atenolol [43], antipsychotic haloperidol [44], but was compatible with desloratadine [40] and diethylcarbamazine citrate [45].

Silicon dioxide is commonly used to improve the flow properties of dry powders in a number of processes, such as tableting and capsule filling, due to its small particle size and large specific surface area. The fluconazole/silicon dioxide (Fig. 3h) curve showed the endothermic peak of the fluconazole melting point at 130.9 °C. This reduction of around 8 °C in the melting was not considered to indicate incompatibility since the DSC curve could be considered as the sum of the individual curves, without the presence of new events or the absence of previously present events. Furthermore, silicon dioxide is commonly used in small amounts in a pharmaceutical formulation and would probably not compromise the product stability. Silicon dioxide was considered compatible with primaquine [46], norfloxacin [32], and ketoprofen [42] but incompatible with enalapril maleate [28].

Sodium lauryl sulfate is an anionic surfactant employed in a wide range of non-parenteral pharmaceutical formulations. It is a detergent and wetting agent effective under both alkaline and acidic conditions. It is also used as an emulsifying agent and tablet and capsule lubricant. Since the use of sodium lauryl sulfate increases the drug

dissolution rate, and thus can affect drug absorption and bioavailability; it is also considered a critical excipient for biowaiver analysis. For the fluconazole/sodium lauryl sulfate (Fig. 3i) mixture the melting event of fluconazole was observed at T_{peak} = 132 °C. The two endothermic events at T_{peak} = 100 °C and T_{peak} = 153 °C, along with the exothermic event at T_{peak} = 167 °C, were also observed for sodium lauryl sulfate alone. It showed interaction with trioxsalen [47], chlorpropamide [48], and levothyroxine sodium pentahydrate [49], but it was compatible with risperidone [41].

In pharmaceutical preparations, mannitol is primarily employed as a diluent (10–90 % w/w) in tablet formulations, where it is of particular value since it is not hygroscopic and thus may be used with moisture-sensitive active ingredients. Administered orally, mannitol is not absorbed in significant amounts from the gastrointestinal tract, but in large doses it can cause osmotic diarrhea [29]. It was found that the use of mannitol led to a lower oral bioavailability of cimetidine compared to sucrose [50]. Because of this gastrointestinal issue it is considered to be a critical excipient [5, 23, 29]. For the fluconazole/mannitol (Fig. 3j) mixture, the DSC curve was considered the superposition of the individual curves, without indication of incompatibility. The fluconazole melting point was observed at T_{peak} = 130.8 °C. Mannitol was compatible with desloratadine [40] and trioxsalen [47] and incompatible with primaquine [46] and omeprazole [51].

Differences were observed in the DSC curves for fluconazole/calcium phosphate dibasic dihydrate and fluconazole/microcrystalline cellulose (Fig. 4).

Microcrystalline cellulose is widely used in pharmaceuticals, primarily as a binder/diluent in oral tablet and capsule formulations where it is used in both wet-granulation and direct-compression processes. In addition to its use as a binder/diluent, microcrystalline cellulose also has lubricant and disintegrant properties which make it useful for tableting [29]. The curve for fluconazole/microcrystalline cellulose (Fig. 4c) showed the displacement of the fluconazole melting point from T_{peak} = 138.2 °C to T_{peak} = 125.6 °C. This decrease of around 13 °C indicates the occurrence of a strong interaction in the solid state, but this does not necessarily correspond to incompatibility.

Microcrystalline cellulose showed interaction with desloratadine [40], enalapril maleate [28], risperidone [41], but it was compatible with venlafaxine hydrochloride [19], sibutramine hydrochloride monohydrate [31], norfloxacin [32], and ketoprofen [42].

Calcium hydrogen phosphate dihydrate is used as a diluent in tablets and capsules. It is also used in pharmaceutical products because of its compaction properties and the good flow properties of the coarse-grade material. The DSC curve for pure calcium phosphate dibasic dihydrate

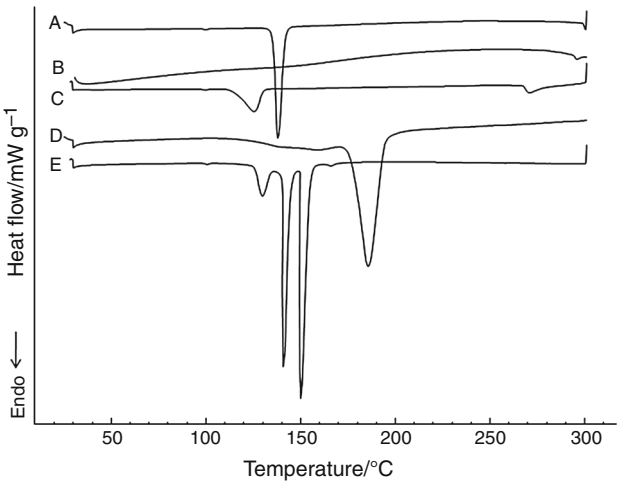

Fig. 4 DSC curves for fluconazole/calcium phosphate dibasic dihydrate and fluconazole/microcrystalline cellulose. *A* pure fluconazole, *B* microcrystalline cellulose pure, *C* fluconazole/microcrystalline cellulose, *D* calcium hydrogen phosphate dihydrate, *E* fluconazol/calcium hydrogen phosphate dihydrate

(Fig. 4d) showed two overlapping endothermic events. The first was a broad peak in the range of 109–169 °C and the second was a sharp peak in the range of 170–206 °C (T_{peak} = 185.4 °C). These events can be attributed to the dehydration (bound water) process. The DSC curve for fluconazole/calcium hydrogen phosphate dihydrate (Fig. 4e) shows three sharp and well-defined endothermic peaks the first event (T_{peak} = 129.7 °C), the second event (T_{peak} = 140.7 °C), and the third event (T_{peak} = 150 °C). In the DSC analysis of the mixture it was not possible to correctly identify the thermal events of each individual compound because it seems that there was a drug–excipient

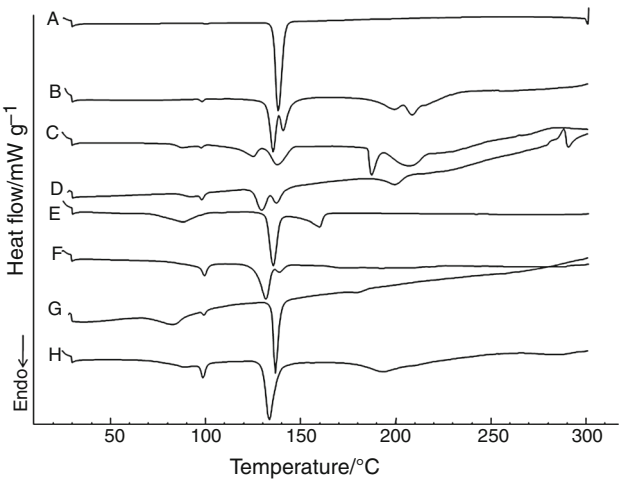

Fig. 5 DSC curves for fluconazole and different commercial formulations. *A* pure fluconazole, *B* reference, *C* generic 1, *D* generic 2, *E* generic 3, *F* similar 1, *G* similar 2, *H* similar 3

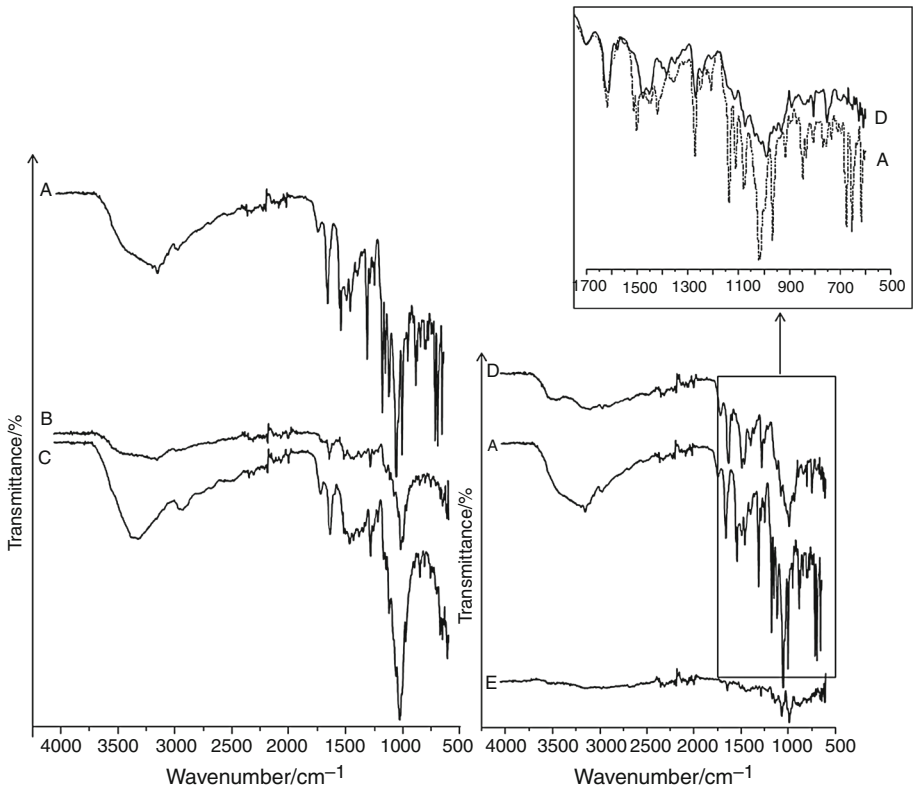

Fig. 6 FTIR spectra of fluconazole (*A*), microcrystalline cellulose (*B*), fluconazole/microcrystalline cellulose (*C*), fluconazole/calcium hydrogen phosphate dihydrate (*D*), calcium hydrogen phosphate dihydrate (*E*)

interaction which altered their thermoanalytical profiles. For this reason, the T_{onset}, T_{peak}, and enthalpy (ΔH) values were not attributed for this mixture (Table 2). Calcium hydrogen phosphate dihydrate was compatible with ciprofloxacin hydrochloride [52] and pefloxacin mesilate [53].

If the drug is compatible with the excipient at high temperatures it is compatible at room temperature. However, if there is incompatibility/interaction at high temperatures it may or may not be incompatible at room temperature. In such cases, additional studies should be performed to investigate the possibility of incompatibility.

The DSC curves for fluconazole and the different commercial formulations are shown in Fig. 5. The thermoanalytical profiles of the formulations differ as expected, since the compositions (excipients) were different, but this did not affect the melting point of fluconazole. Even the G3 formulation which appeared to be manufactured by wet granulation presented a similar DSC curve to the other formulations. In fact, the thermal behavior of fluconazole was maintained in all analysis of the commercial pharmaceutical formulations with microcrystalline cellulose (G3 and S2) and calcium hydrogen phosphate dihydrate (S2).

For all formulations, thermal events were observed up to 100 °C. They were considered as loss of surface (humidity) water. They could also be attributed to magnesium stearate and/or sodium lauryl sulfate; however, these excipients are usually present at low concentration in formulations (about

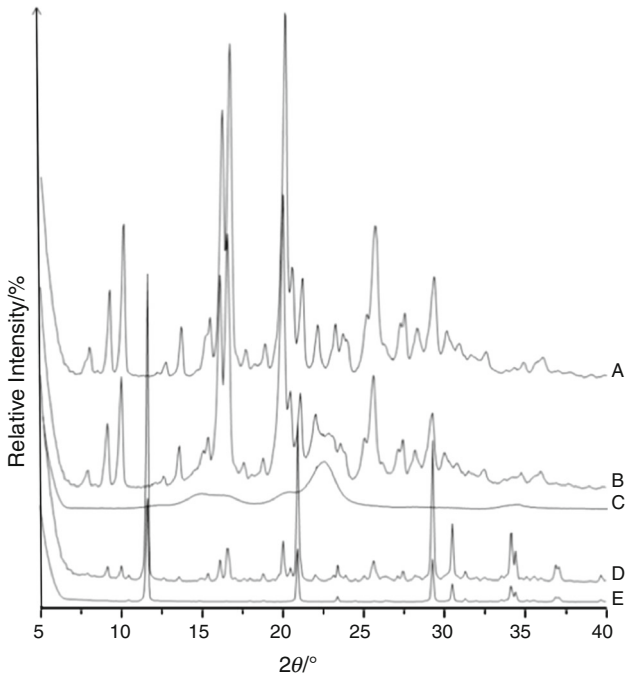

Fig. 7 Fluconazole (*A*), fluconazole/microcrystalline cellulose (*B*), microcrystalline cellulose (*C*), fluconazol/calcium hydrogen phosphate dihydrate (*D*), calcium hydrogen phosphate dihydrate (*E*)

0.5–2 %) hindering their identification. Thermoanalytical profile of R, G1, G2, and S1 (Fig. 5b, c, d, and f, respectively) showed two overlapped peaks in the range of

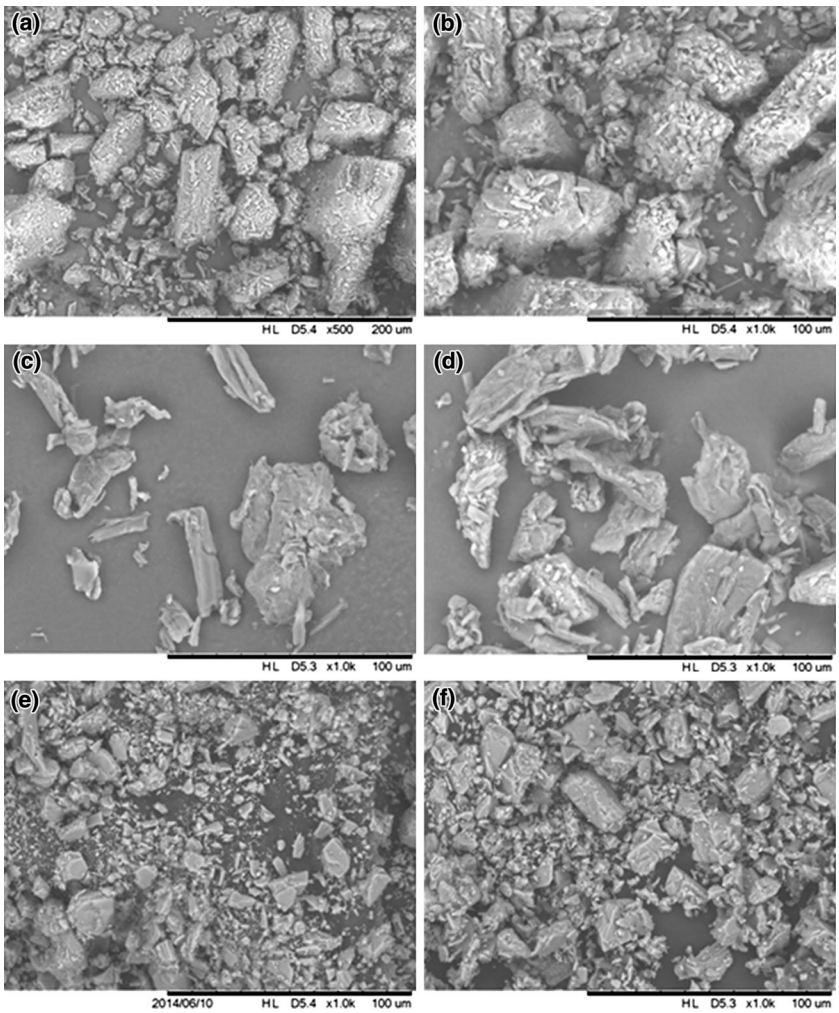

Fig. 8 SEM photomicrographs of: fluconazole (×500) (**a**), fluconazole (×1,000) (**b**), microcrystalline cellulose (×1,000) (**c**), fluconazole/microcrystalline cellulose (×1,000) (**d**), calcium hydrogen phosphate dihydrate (×1,000) (**e**), fluconazol/calcium hydrogen phosphate dehydrate (×1,000) (**f**)

125–145 °C that could be explained by the fluconazole melting followed by the dehydration of lactose monohydrate. Probably, due to the influence from the excipients these two events were visualized in the formulations curves and not in the binary mixture curve (Fig. 3c). The events observed for R, G1, G2, S2, and S3 (Fig. 5b, c, d, g, and h, respectively) in the range of 185–230 °C could be due to lactose melting. The endothermic event observed at 160 °C for G3 is explained by the presence of mannitol (Fig. 5e).

Fourier transform infrared spectroscopy (FTIR)

The interaction of fluconazole with microcrystalline cellulose and calcium hydrogen phosphate dihydrate was observed in binary mixtures by DSC. Thereby, the FTIR spectra of these mixtures were obtained in order to identify a possible chemical interaction (Fig. 6). For fluconazole, the vibrations of the various functional groups present in the molecule could be attributed to a broad band due to hydrogen bonded O–H stretching vibrations in the range of 3,600–2,500 cm^{-1}; 1,619 and 1,514 cm^{-1} bands due to C=C stretch aromatic ring; 1,502 and 1,420 cm^{-1} bands due to triazole ring stretch; 1,273 cm^{-1} for C–F stretch; 1,138 cm^{-1} for triazole ring breathing; 1,020 cm^{-1} for C–H aromatic ring; 967 and 846 cm^{-1} for C–H triazole ring [54, 55].

In the mixture fluconazole/microcrystalline cellulose, there was a shift of the peak of the 3,600–2,500 band from 3,116 to 3,286 cm^{-1}, compared to the spectrum of pure fluconazole. This is possibly due to greater interaction strength of hydrogen bonds. The other absorption bands can be considered as the superposition of the individual ones without absence, shift, or broadening in the vibration bands of fluconazole. In the mixture fluconazol/calcium hydrogen phosphate dihydrate some changes were observed the disappearance of some peaks at 1514, 1502, 1420, 1138, 967, and 846 cm^{-1}. Most of the bands that disappeared are associated with the triazole ring, which is responsible for the antifungal activity of fluconazole, thus an interaction or incompatibility with this excipient may impair the clinical efficacy of the drug.

XRPD

XRPD studies were performed in order to obtain more information regarding the crystalline characteristics of the binary mixtures of fluconazole, microcrystalline cellulose, and calcium hydrogen phosphate dihydrate. The 2θ values of the diffraction peaks (Fig. 7) for fluconazole were $2\theta = 9.27°$, 10.13°, 16.23°, 16.68°, 20.09°, 21.20°, 25.67°, and 29.33°. For both binary mixtures only small changes in the peaks intensities were observed, which did not indicate any change in the crystalline structure of the compounds.

SEM

The photomicrographs obtained by SEM (Fig. 8) did not evidence any interaction between fluconazole and the excipients. The SEM images have shown that both fluconazole and excipients particles maintained their morphology, and the drug crystals appeared dispersed on the surface of excipients particles. The SEM data were in agreement with the XRPD, where no changes in the structure and/or incompatibility were observed.

Conclusions

Thermoanalytical techniques, mainly DSC, have been increasingly used in the characterization of solid state interactions and early detection of drug–excipient compatibility. These pre-formulation studies are an important step to obtaining a reliable and effective pharmaceutical formulation. However, in the development of formulations which are candidates for the BE biowaiver they are even more critical since these formulations will not be evaluated in vivo. The composition analysis verified that the critical excipients were not present in any of the formulations, which is a prerequisite for the biowaiver. The interaction of fluconazole with microcrystalline cellulose and calcium hydrogen phosphate dihydrate was observed in binary mixtures, but was not confirmed in the analysis of the commercial formulations and by FTIR, XRPD, and SEM. DSC proved to be an important technique in the first step of product development. If interactions are detected, future problems in the dissolution and/or stability can be foreseen. The assessment of API–excipient interactions can be considered even more critical for medicines which are candidates for the BE biowaiver, since they will not be submitted to in vivo studies.

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