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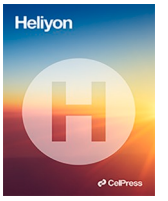

Research article

Impact of crystal polymorphism of rifaximin on dissolution behavior

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ABSTRACT

Introduction: Rifaximin is an intestinal antiseptic which has five (pseudo) polymorphs α, β, γ, δ and ε. These last (pseudo)polymorphs have different physicochemical properties. The objective of the study is to assess the impact of rifaximin polymorphism on its dissolution rate which could affect its bioavailability.

Material and methods: The analytical validation of dissolution assay method by UV–Visible spectrophotometry was carried out according to ICH Q2. The physicochemical characterization (solubility test, FTIR, DSC, XRD) was carried out on four active pharmaceutical ingredient (MP1, MP2, MP3, MP4). MP1 and MP2 were used by the manufacturer of generic brand 1 (G1) and MP3 and MP4 were used by the manufacturer of generic brand 2 (G2). The comparative in-vitro dissolution study was carried out on the leader brand (P), G1 and G2.

Results: The four MPs were analyzed by XRD. The results of analysis showed that MP1 and MP4 were a mixture of α form and amorphous form. MP2 had an amorphous form and MP3 had a crystalline form β. The spectra of FTIR showed that the four MP had characteristics bands of rifaximin in the domain 4000-400 cm^{-1}. The differences between the spectra of the four MPs were observed among the amorphous form (MP2), around the region 1800 to 1820 cm^{-1} which is attributed to the vibration of the CO group. An additional difference observed among the amorphous form (MP2) is around the region 1400 cm^{-1} which is attributed to the banding OH. The thermograms of MP1, MP2 and MP4 showed endothermic peaks which are probably attributed to the departure of water which indicate that MP1, MP2 and MP4 are pseudopolymoph (hydrate). For the four MPs, probably the melting points are interrupted by the phenomenon of phase transformations (Crystallization) which are reflected by exothermic peaks around 200°C–250 °C.Our results showed that the crystalline polymorphism of rifaximin influences its solubility. According to the results of the solubility test, the β crystal form of rifaximin (MP3) had the lowest solubility (3.47 μg/ml). MP2 had the highest solubility (8.35 μg/ml) and MP1 and MP4 had intermediate solubilities (5.47 μg/ml and 6.74 μg/ml). Comparative in vitro dissolution results showed that the dissolution profile of P was not similar to that of G1 and G2 (% dissolution $(P)_{30min}$ = 60%; % dissolution $(G1)_{30\ min}$ = 100% and % dissolution $(G2)_{30\ min}$ = 115%; f1(P versus G1) = 44; f1(P versus G2) = 61) in M1, while G1 and G2 had comparatively similar dissolution profiles (% dissolution $(G1)_{30\ min}$ = 100%; % dissolution $(G1)_{30\ min}$ = 110%; f1 (G1 versus G2) = 14) in M1.

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Table 1
Qualitative composition of the three brand medicines of rifaximin.

	Leader brand (Normix®) 200-mg film-coated tablet	Generic brand 1 200-mg film-coated tablets	Generic brand 2 200-mg film-coated tablets
Active ingredient	Rifaximin		
Excipients	Sodium starch glycolate, Glycerol distearate, Colloidal anhydrous silica, Talc, Microcrystalline cellulose	Pregelatinized Corn Starch, Microcrystalline Cellulose, Colloidal Silica Anhydrous, Magnesium Stearate, Talc	Corn starch, sodium laurysulfate, polyvidone, magnesium stearate
Coating components	Hypromellose, Titanium dioxide, EDTA disodium, Propylène glycol, Disodium edetate, Propylene glycol Red iron oxide E172	Hypromellose, titanium dioxide, Polyethylene glycol/Macrogol Red iron oxide, yellow iron oxide	Hypromellose, titanium dioxide, Polyethylene glycol/Macrogol Red iron oxide, yellow iron oxide

Conclusion: This study highlighted the impact of rifaximin polymorphism on its physico-chemical properties (crystal structure, thermal behavior, solubility) and on its dissolution behavior which could affect the rifaximin bioavailability.

1. Introduction

Intestinal antiseptics, among others rifaximin, are designed to act locally in the gastrointestinal tract. Variations in local and systemic bioavailability due to formulation changes may influence the efficacy and safety of locally acting drugs. In addition to formulation, physicochemical characteristics of active pharmaceutical ingredient (API) have a significant influence on systemic bioavailability. Indeed, chemical structure, polar surface, pH distribution, particle size, salt form, drug complexation, as well as the crystalline forms can affect the dissolution and absorption rates of molecules. While most of the above parameters have been widely studied for locally acting drugs, crystalline polymorphism has often been overlooked [1].Polymorphism is "the possibility that have molecules to exist under at least two distinct crystalline forms". Different polymorphs of a drug may exhibit different physicochemical properties, including stability, melting point, reactivity, dissolution rate, and solubility, which may affect the pharmacokinetics and pharmacodynamics of a drug [1].

The crystalline polymorphism exhibited by several API had attracted a lot of attention since the first report by Aguiar et al. showing the effect of crystal polymorphism on the absorption of chloramphenicol [2].Different polymorphs of API might have different physical-chemical properties, including stability, reactivity, dissolution rate and solubility which might affect the pharmacokinetics and pharmacodynamics of drug. Relevant examples of the impact of polymorphism on systemic bioavailability have been reported [1, 3]. In extreme cases, a polymorph might even be ineffective, as happened with polymorph II of ritonavir [4]. For this reason, it was recommended to pay particular attention to this point during the drug formulation step. Unfortunately, the problem of polymorphism had often been neglected in the past.

Rifaximin is an intestinal antiseptic used orally. It is used for the treatment of hepatic encephalopathy, traveler's diarrhea, irritable bowel syndrome, *Clostridium difficile* infections, ulcerative colitis and acute diarrhea [5].Rifaximin has the phenomenon of polymorphism. It has five known polymorphs (pseudo) α, β, γ, δ and ε [1,6]. The crystalline form having a minimal systemic absorption, is α form. The latter is significantly different from the amorphous form. The different polymorphs of API have different physicochemical properties in the solid state (melting point, solubility, stability and dissolution). This has an influence on the pharmacokinetic properties of the drug. Solid state control is essential because the polymorphism problem can occur in API production step or storage step. The polymorphic transition can occur at different stages and can influence the efficiency and safety of the finished product [7].

The objective of the present work was to study the impact of rifaximin crystalline polymorphism on its solubility and dissolution rate which are two key factors that impact the absorption rate of rifaximin and hence could affect its bioavailability. As a result, the efficiency and safety of rifaximin medicines could be altered.

2. Material and methods

2.1. Reagents and active pharmaceutical ingredients (MP)

Rifaximin of 99.8% purity (Reference substance) was kindly provided from LNCM.MP1 and MP2 were kindly provided from the manufacturer of generic brand 1 (G1).MP3 and MP4 were kindly provided from the manufacturer of generic brand 2 (G2). Regarding the leader brand (P), we didn't have available the MP of P. A priori, we didn't know the different polymorphs of MP1-4.

Monosodium phosphate NaH_2PO_4 was purchased from Novachim laboratories, Na_2HPO_4 disodium phosphate was purchased from Alpha Chemika laboratories and sodium dodecyl sulphate was purchased from Sigma Aldrich laboratories. orthophosphoric acid (85%) was purchased from laboratories Carlo Erba. A purified water freshly prepared was used.

The phosphate buffer solution pH = 7.4 (1 L) was prepared as follows: 2.62 g of NaH_2PO_4 and 11.5g of Na_2 HPO_4 dissolved in 1 L of water in a volumetric flask. Buffer media at pH = 4.5 and 6.8 were prepared from phosphate buffer solution at pH = 7.4 whose pH was adjusted using orthophosphoric acid.

2.2. Reagents and pharmaceutical samples

The leader brand of rifaximin P and the generic brand of rifaximin (G1, G2) were purchased amongst the brand medicines available in the Tunisian pharmacy at the time of the study.

The brand medicines have the form of 200-mg film-coated tablets. The qualitative composition of these three brand medicines was reported in Table 1.

The rifaximin dissolution assay was performed by UV–visible spectrophotometry using the Shimadzu 1650 PC UV–visible spectrophotometer (Japan). The choice of maximum wavelength (232 nm) was fixed after performing a spectral scanning 200 nm–400nm. The analytical validation was carried out according to the recommendations of ICH Q2 (R1) (International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use ICH,2015).

The validation parameters and acceptance criteria are shown in Table 2.

2.3. Physico-chemical characterization of MP

The physicochemical characterization (solubility, DSC, FTIR, XRD) was carried out on four active pharmaceutical Ingredients (MP1, MP2, MP3, MP4) comes from four different suppliers. MP1 and MP2 were used by the manufacturer of generic brand 1 (G1) and MP3 and MP4 were used by the manufacturer of generic brand 2 (G2).

2.4. Fourier transform infrared spectroscopy (FTIR) analysis

The analysis of the four MPs was carried out using a PerkinElmer FTIR spectrophotometer (model 100, Japan), using a KBr pellet method (3 mg rifaximin is mixed with 297 mg KBr in an agate mortar and compressed into fine pellets ranging from 400 to 4000 cm^{-1} with a resolution of 1 cm^{-1}). Data analysis and tracing of the IR spectra corresponding to the four MPs is done using the PerkinElmer spectrum software V6.3.5.

2.5. Differential scanning calorimetry (DSC) analysis

Differential scanning calorimetry was performed with a DSC Metler Toldeo (model 823 E). Mass samples between 1 and 2 mg were weighed into aluminum cups and analyzed over a temperature range of 30 °C–300 °C with a heating rate of 10 °C/min. The resulting data was processed using Metler Toldeo Star software SW 9.01.

2.6. X-ray powder diffraction analysis

The analysis of the four MPs was carried out using a Panalytical X'pert automated diffractometer equipped with a goniometer (theta-2 theta) in the Bragg-Brentano geometry by reflection (fixed sample, detector and source moving). The incident beam collimation was in the fixed mode. The equipment was equipped with a graphite monochromator. The Cu anode was used as an X-ray source at 45 kV and 40 mA. The wavelength used was that of copper Kα1/Kα2 (1.54060/1.54443 nm). The diffractograms were recorded in the 3–70° angular range with a counting step of 0.02 (2 theta). Acquisition times of 12.28 s per step and an opening angle of the fixed slots equal to 0.25°. The measurements were made at 25 °C. The treatment of the X-ray diffraction data was done using Panalytical's XPert High Score Plus software (version 4.6a).

2.7. Study of solubility

The solubility of the different MP was determined as follows: for each MP, a sample corresponding to 50 mg of rifaximin was weighed and placed in a 10 ml volumetric flask of distilled water. The sample was placed under magnetic stirring (250 rpm) for 24 h at room temperature. Then, let stand for 2 h at the same temperature. The supernatant was removed using a syringe and filtered through a 0.45 μm porosity filter and then read without dilution by Thermo Scientific UV–visible spectrophotometry (Evolution 60 model) at 232 nm. The test is repeated 3 times. The solubility results are expressed as the average of these repetitions ± standard deviation ($n = 3$). The comparison between mean solubility values of the four MPs was performed using student test using the Excel version 2007 software. The observed difference between the two groups was considered significant for $p \leq 0.05$.

2.8. Dissolution test

The comparative in-vitro dissolution study was carried out on the leader brand medicine P and the two generic brand medicines G1 and G2 marketed in Tunisia.

The dissolution tests were carried out in accordance with the recommendations USP. The apparatus used is AT Sotax equipped with

Table 2
Validation parameters and acceptance criteria of rifaximin assay method by UV–Visible spectrophotometry.

Validation parameters	Tests	Acceptance criteria
Selectivity (standard addition method)[a]	Slope comparison test	No interference at the maximum wavelength = 232 nm
Linearity	Correlation coefficient	$R \geq 0.999$
	Comparing the y-intercept with zero	t-Student test
Precision	Coefficient of variation of repeatability (CVr)	CVr <5%
	Coefficient of variation of reproducibility (CVR)	CVR <10%
Accuracy	Recovery values	The mean recovery value and individual recovery value should obtain 97%–103%
	Recovery confidence interval	100 % should be in the recovery confidence interval

[a] the standard addition method was done by crashing the rifaximin tablets 200 mg for every brands (leader brand, generic brand 1, generic brand 2) and by adding precise amounts of rifaximin to every samples (10%, 15%, 20%, 25%) which allow to have series of standards.

paddle stirrers. In vitro release study was performed using two media: the first media has a pH equal to 4.5 and contained 0.375% of SLS and the second media has a pH equal to 6.8 and contained also 0.375% of SLS. The Volume of dissolution media is equal to 1000 ml and the temperature of the media is equal to 37 ± 0.5%. The rotation speed is equal to 75 RPM/min.

The test was carried out over 120 min with samples taken at times t10, t20, t30, t45, t60, t90 and t120 min. The samples were diluted appropriately and then analyzed by UV–visible spectrophotometry at 232 nm. The results obtained were expressed as percentage of dissolution and represent the mean ± standard deviation of 6 repetitions (n = 6).

2.8.1. Calculation of similarity (f1) and difference (f2) factors

The mathematical comparison was performed by applying f_1 [Equation (1)], and f_2[Equation (2)].[8] The values of $f_1$between zero and 15 and f_2 between 50 and 100 ensure the identity or equivalence of the two dissolution profiles. In both equations, R and T represent the dissolution percentages of the leader brand and the generic brand, respectively:

$$f_1 = \left\{ \left[\sum_{i=1}^{p} |R\text{-}T| \right] \Big/ \left[\sum_{i=1}^{P} R \right] \right\} \times 100 \quad (1)$$

()

$$\left\{ \left[1 + \left(\frac{1}{P} \right) \sum_{i=1}^{P} (R - T)^2 \right]^{1/2} \times 100 \right\} \quad (2)$$

3. Results

3.1. Physico-chemical characterization of MPs

3.1.1. FTIR infrared spectroscopy

The superposition of the spectra of the four MP showed the existence of characteristic bands of rifaximin in the domain 4000-400 cm^{-1}. In particular, a broad band due to the vibration of the bonds OH associated with 3450 cm^{-1}. The spectrum also showed a band around 2900 cm^{-1} corresponding to the stretching vibrations relating to the CH bond of rifaximin. A light band around 1550 cm^{-1} referred mainly to the secondary amines of this molecule. finally, we also observed a characteristic band of the carbonyl function towards 1650 cm^{-1} (Fig. 1). The differences between the spectra of the four MPs are observed among the amorphous form (MP2) around the region 1800 to 1820 cm^{-1} which is attributed to the vibration of the CO group. The significant intensity of the band CO indicates that the CO of amorphous rifaximin (MP2) are free and not engaged to the H band of OH group. However, the MPs which have mostly the crystalline form (MP1, MP3, MP4) didn't have an important vibration of the CO group given this last is engaged to H band of OH group because the ordered structure of the crystalline forms allows easily this kind of band. An additional difference between the amorphous form (MP2) and the others forms (MP1, MP3, MP4) is observed around the region 1400 cm^{-1} which is attributed to the banding OH due to the fact of absence of hydrogen bands between molecules given the disorderly amorphous form which not allows this type of band. Consequently, many OH are free which increase the vibration intensity of this group.

3.2. Thermal analysis of the four MPs of rifaximin by differential scanning calorimetry (DSC)

The thermograms of MP1 and MP4 showed respectively remarkable endothermic peaks around 58.15 °C and 57.32 °C which are probably attributed to the departure of water which indicate that MP1 and MP4 are pseudopolymoph (hydrate).

The thermogram of MP2 showed a small endothermic peak around 55.5 °C which also attributed to the departure of water. However, this form of rifaximin contained a few amount of water.

The thermogram of MP3 didn't exhibit an endothermic peak around 50 °C which can be explained by the fact that this form of rifaximin didn't contain water in its structure and it is not a hydrate.

The melting point of rifaximin is usually above 200 °C [9]. For the four MPs, probably the melting points are interrupted by the

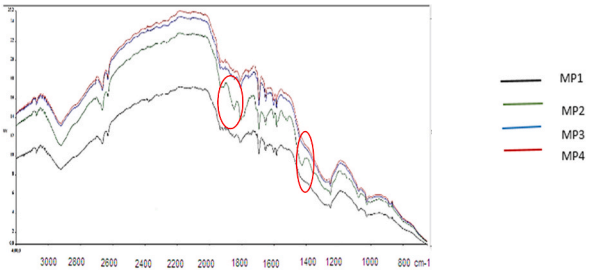

Fig. 1. FTIR spectroscopy analysis of the four MPs.

phenomenon of phase transformations which are reflected by exothermic peaks. For MP1 and MP4, the exothermic peaks are observed around 204–225 °C. Regarding MP2, the exothermic peak is observed around 200–250 °C and finally the MP3 exhibited an exothermic peak around 199–230 °C.

The intensive exothermic peaks observed around 300 °C for all MPs are explained by the decomposition and the degradation of rifaximin due to the intensive heating. Fig. 2 showed the different DSC thermograms of the four rifaximin MP.

3.3. Characterization of rifaximin polymorphs by X-ray diffraction

Fig. 3 illustrated the different diffractograms of the four MP studied. In fact, the diffractograms 1 and 4 corresponded to a mixture of α form and amorphous form. Indeed, we found the characteristic angles of form α (6.6°,7.4°, 7.9°, 8.8°). The diffractogram 2 corresponds to amorphous form since there is no characteristic diffraction angle. The diffractogram 3 corresponds to crystalline form β.In fact, we found the characteristic diffraction angles of β-form (5.4°,6.4°, 7.0°, 7.8°, 9.0°, 10.4°) [10].The manufacturer of brand leader medicine P had used the α form of rifaximin [11].The characteristic diffraction angles of each MP were shown in Table 3.

3.4. Study of solubility of the four MPs

The solubilities of MP1, MP2, MP3 and MP4 were respectively equal to 5.47; 8.35; 3.47 and 6.74 μg/ml. There was a significant difference in solubility between MP1 and MP2 ($p = 0.00217 < 0.05$); between MP1 and MP3 ($p = 0.037$); between MP2 and MP3 ($p = 5.7253\ E^{-5}$) and between MP3 and MP4 ($p = 0.031$).

The results of solubility study of the four MPs were illustrated in Fig. 4.

3.5. Dissolution test

The results of the in vitro dissolution study of the two generic brands and the leader brand were illustrated in the figures below. The comparative dissolution profile in a phosphate buffer at pH = 6.8 supplemented with SLS 0.375% (Fig. 5) exhibited the lowest

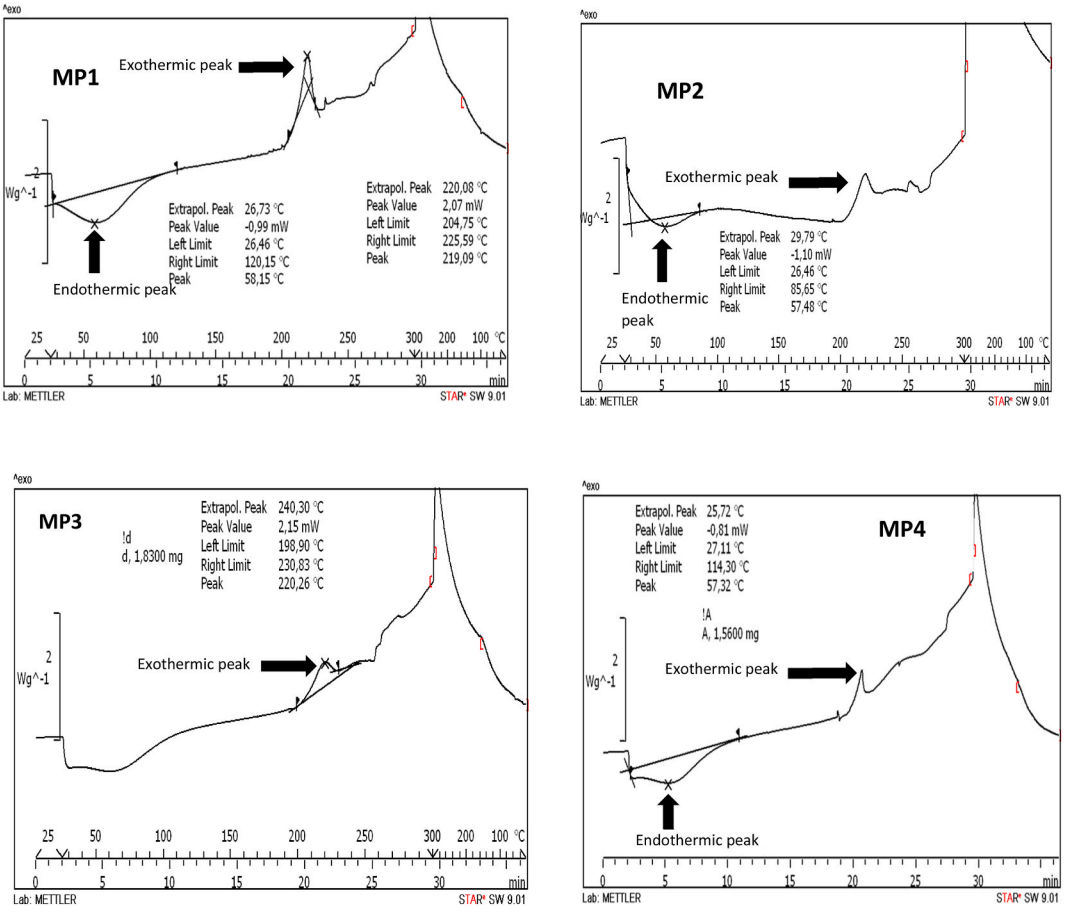

Fig. 2. DSC thermal analysis of the four MPs.

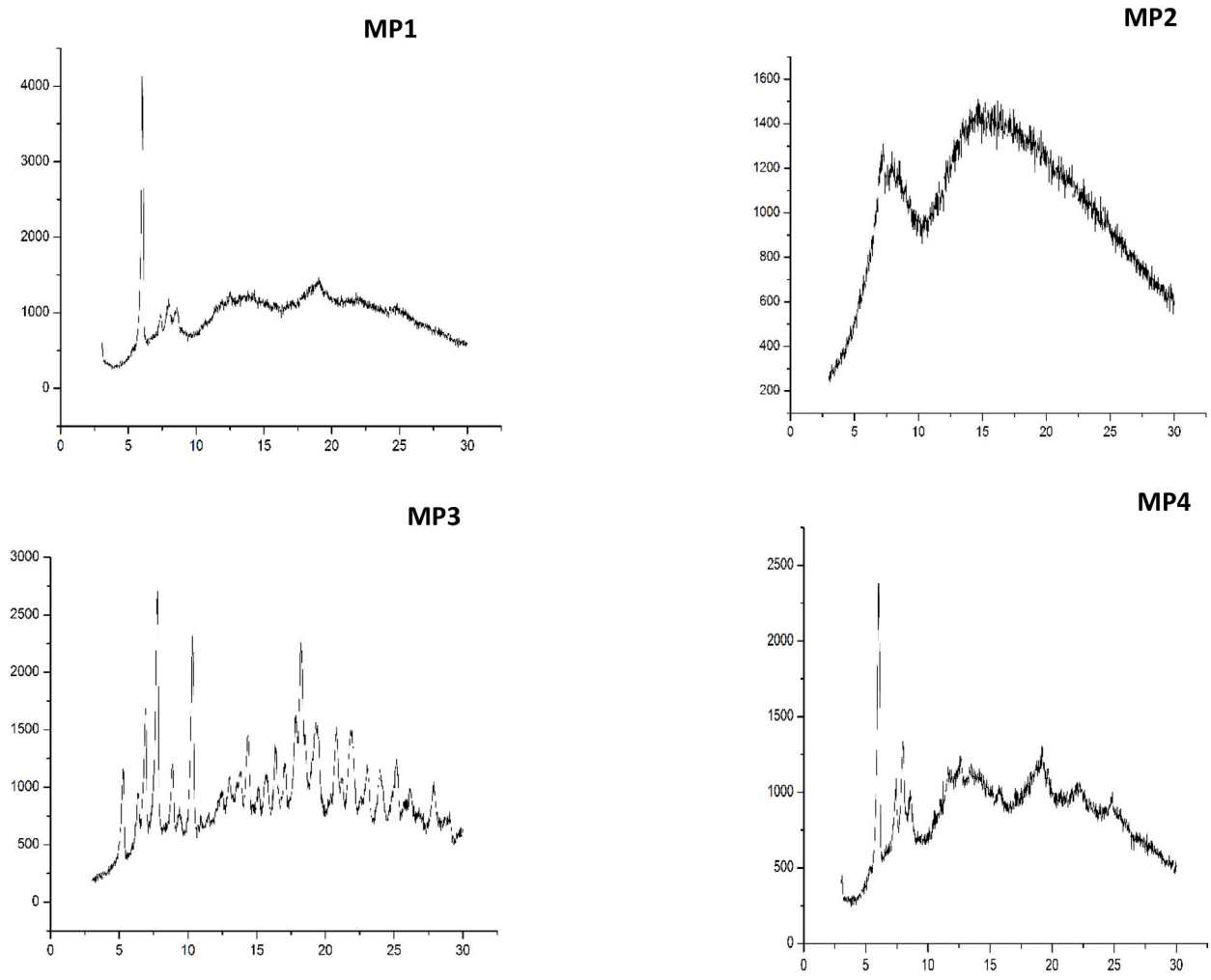

Fig. 3. X-ray diffractograms of MP1, 2, 3 and 4.

Table 3
Characteristic diffraction angles of the four MP.

MP	Characteristic diffraction angles (2 theta)
MP1	6.006°; 7.35°; 7.95°; 8.506°
MP2	–
MP3	5.2941°; 6.334°; 6.9186°; 7.794°; 8.9081°; 9.3644°; 10.3317°
MP4	6.01°; 7.41°; 7.95°; 8.51°

dissolution rate for the leader brand medicine P (% dissolution $_{30min}$ = 60%). However, the generic brands G1 et G2 exhibited the highest dissolution rate (dissolution $_{30min}$ = 100% and 115% respectively for G1 and G2). Similarly, the comparative dissolution profile in a phosphate buffer at pH = 4.5 supplemented with SLS 0.375% (Fig. 6) exhibited the lowest dissolution rate for the leader brand P (% dissolution $_{30min}$ = 60%) and the generic brands G1 et G2 exhibited the highest dissolution rate. (dissolution $_{30min}$ = 100% and 115% respectively for G1 and G2).

The comparison between the leader brand of rifaximin and the generic brand G1 in medium M1 and M2 showed that the difference in comparative dissolution profiles was significant in the medium M1, M2 ($f_1 = 44$, $f_1 = 46$; $f_2 = 25$, $f_2 = 24$ respectively).

The comparison between the leader brand of rifaximin and the generic brand G2 showed that the difference in comparative dissolution profiles was significant in the M1 and M2 media ($f_1 = 61$, $f_1 = 56$; $f_2 = 19$, $f_2 = 20$, respectively).See Table 4.

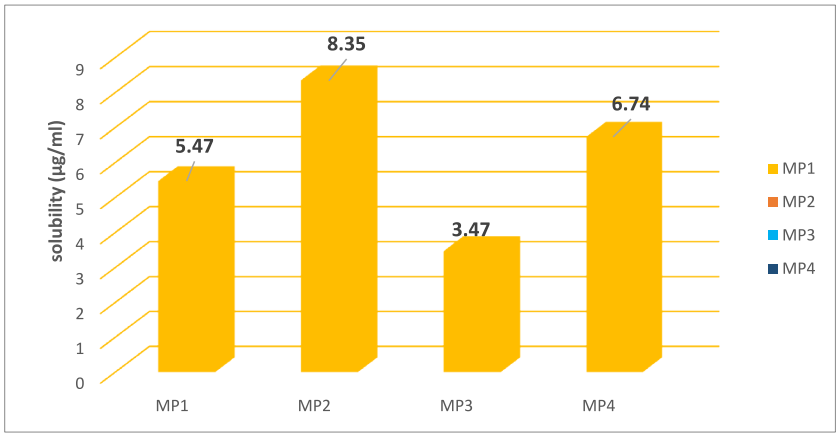

Fig. 4. Study of MP solubility in water (stirring speed = 250 rpm/24 h at room temperature).

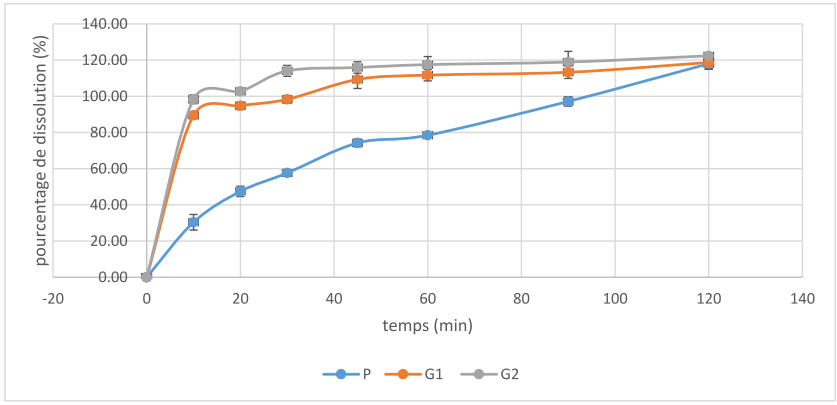

Fig. 5. Comparative dissolution profile in a phosphate buffer pH = 6.8 supplemented with SLS 0.375%.

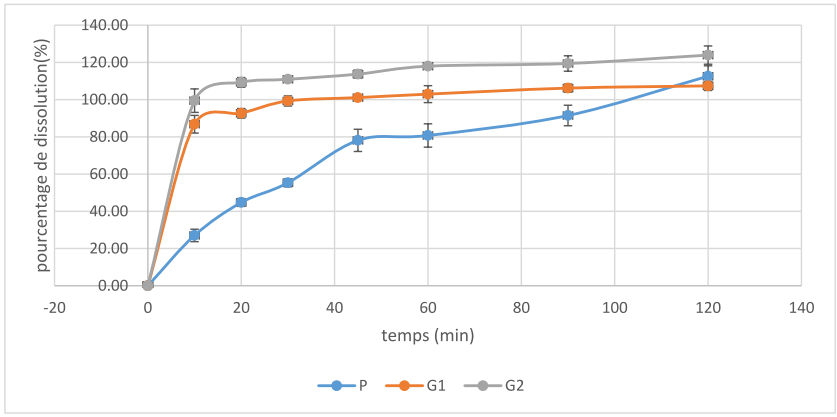

Fig. 6. Comparative dissolution profile in a phosphate buffer pH = 4.5 supplemented with SLS 0.375%.

4. Discussion

The aim of our work was to study the impact of crystalline polymorphism on the solubility and the dissolution rate of rifaximin and thus on its bioavailability. For this purpose, we studied the physicochemical properties of four MPs used by two manufacturers of generic brands (IR-FT, DSC, XRD, solubility). MP1 and MP2 were used by the manufacturer of G1 and MP 3 and MP4 were used by the manufacturer of G2. Then, we carried out a comparative dissolution study of these two generic brands and the leader brand medicine.

Table 4
Difference factor and similarity factor of the generic brands G1 and G2 against the leader brand P in M1 medium and M2 medium.

M1 medium[a]			
	P vs G1	P vs G2	G1 vs G2
f1	44	61	14
f2	25	19	51
M2 medium[b]			
f1	46	56	7
f2	24	20	61

[a] phosphate buffer pH = 4.5 added with 0.375% SDS.
[b] phosphate buffer pH = 6.8 with 0.375% SDS.

Macroscopically, the four MPs showed significantly different colors (yellow orange for MP4 and red orange for MP1, MP2 and MP3).

The DSC curves of the four MPS showed various peaks at different temperatures: endothermic peaks around 50 °C that appeared at different temperatures (58.15 °C, 57.32 °C and 55.5 °C, respectively for MP1,MP4 and MP3). These peaks were attributed to water loss which could confirm that MP1, MP4 and MP3 were considered as pseudopolymorphs (hydrates). However, MP2 didn't exhibit this endothermic peak around 50 °C, hence it's not a hydrate. The four MPs exhibited different exothermic peaks around 200–250 °C which could be correspondent to phase transformations (crystallization).The temperature range 200°C–220 °C could be attributed to the range of melting point of rifaximin polymorphs [12] but it's hidden by phase transformations. DSC had shown that the four MPs had significantly different water loss temperatures and different temperatures of phase transformation (crystallization), suggesting that they had different polymorphs. Our study were in agreement with the study of kogawa AC et al. [12] which found that the different polymorphs of rifaximin had different thermal behavior among other things melting points.

The four MPs were analyzed by XRD. The results of the analysis showed that MP1 and MP4 were a mixture of α form and amorphous form. MP2 had an amorphous form and MP 3 had a crystalline form β. Study by Bragga et al. [13] had also described several polymorphs of rifaximin (α, β, γ, δ, ε). The study by Braga et al. [13] revealed that the polymorphs of rifaximin were hydrates and the addition or elimination of water might lead to changes in polymorphism. Particular attention must be paid to the handling and storage of the drug (either the leader brand or the generic brand) to ensure the stability of the desired crystal structure [11].

Our results showed that the crystalline polymorphism of rifaximin influenced its solubility. According to the results of the solubility test, the β crystal form of rifaximin (MP3) had the lowest solubility. The amorphous form (MP2) had the highest solubility and the α-form and amorphous form mixtures (MP1 and MP4) had intermediate solubilities. These results were in agreement with the literature. Indeed, the amorphous form always had greater solubility and dissolution rate than that of the crystalline form. This could be explained by the fact that the amorphous form had a disordered molecular arrangement and had a free energy greater than that of the crystalline state, which led to a greater and faster solubility and dissolution of the amorphous state compared to the crystalline state [14].

Comparative dissolution results in vitro showed that the dissolution profile of the leader brand was not similar to that of the two generic brands G1 and G2 while the two generic brands G1 and G2 had comparatively similar dissolution profiles. This was explained by the fact that the leader brand had a crystalline form of rifaximin (α-rifaximine) [1,11].whereas, probably according to the physicochemical characterization results and the results of in vitro comparative dissolution test, G1 had the amorphous form or a mixture of the α form and the amorphous form (MP2, MP1) and G2 had a mixture of the α form and the amorphous form (MP4). Our results were consistent with the study from Blandizzi et al. [1,11].The latter found that the in vitro comparative dissolution profiles, made with identical formulations containing respectively the amorphous rifaximin and the polymorph α were not similar. Pharmacokinetics studies reported in the literature clearly showed that the amorphous form had a higher bioavailability compared to polymorph-α. This is reflected by a higher concentration (Cmax), an increased area under the curve (AUC) and urinary excretion. This difference was evident with both dosages (200 and 550 mg), the higher the dose, the higher the ratios observed. Since the maximum dose was administered at daily doses of 800–1200 mg, systemic absorption of the amorphous form appeared to be considerable [1]. Indeed, the systemic passage of rifaximin could be explained by the fact that the oral absorption of rifaximin had additional complexity, because it was limited by the efflux transporter glycoprotein-P (P-gp) [15]. In this article [16], they were able to reveal that there was a threshold level of supersaturation of rifaximin which was related to the saturation of the transporter P-gp, above this threshold a fraction of rifaximin dose could escape from efflux transport, and overall permeability was increased. In another study by Blandizzi et al. [11], it was found that the pharmacokinetics profile of generic brand of rifaximin differed significantly from that of the leader brand medicine.

Since the XRD spectra showed that the generic brands contained a mixture of amorphous and crystalline form, it was conceivable that the systemic bioavailability of rifaximin was dependent on the composition (in terms of crystalline form and amorphous form) present in the formulation. In our case, the differences observed between the leader brand and the generic brand could not be attributed solely to differences in the composition of excipients, since those used in the formulation of the leader brand and the generic brand were virtually the same, but also to the phenomenon of polymorphism. Overall, it was possible that for the generic brands tested, the manufacturing processes or the storage conditions were different from those of the leader brand. Solubility and dissolution rate are two well-known key factors that influence obviously the absorption rate through gastrointestinal barrier and hence the bioavailability of rifaximin. Indeed, the differences in solubility and dissolution profiles among the different polymorphs of rifaximin could lead to the increased systemic bioavailability of the drug containing the amorphous form that could raise some clinically relevant concerns. In fact, for a poorly absorbed antibiotic, whose antimicrobial activity was intended to be exerted in the gastrointestinal tract, systemic

absorption could lead to reduced local bioavailability and potentially to systemic adverse effects [17]. The risk of adverse effects correlated with blood levels [18]. With the crystalline form (α-rifaximin), short- and long-term tolerance was extremely good [17,19], probably due to the lack of systemic absorption. However, the use of rifaximin containing polymorphic forms other than the α-form, in particular the amorphous form, could lead to quite significant adverse effects when taken in the medium or long term. This was the example of treatment of SIBO(bacterial proliferation of the small intestine) [20].In addition, a more alarming adverse event is the development of resistance to anti-tuberculosis [11]. This was particularly relevant for *Mycobacterium Tuberculosis* and *Neisseria Meningitides* because rifampicin (another member of the rifamycin family) was an essential antibiotic in the treatment of tuberculosis and the prevention of *Neisseria meningitis* [11]

The limitations of the study are the lack of permeability studies and the bioavailability study. In the future, we suggest completing our research by performing permeability studies and conducting bioavailability study to confirm the conclusions of our research.

5. Conclusion

Rifaximin is a synthetic derivative of rifamycin with intestinal antiseptic action. It is an antibiotic designed to act locally in the gastrointestinal tract. Any systemic bioavailability of this molecule may cause adverse effects, inefficiency and even problems of cross resistance with rifampicin. Rifaximin has the phenomenon of crystalline polymorphism that can influence its pharmacokinetics and pharmacodynamics. The control of crystalline polymorphism during the synthesis of API is a major challenge for the pharmaceutical industry. Indeed, depending on the selected polymorphic form, the properties of the developed medicine may be totally different.

The XRD analysis of the four MPs showed that MP1 and MP4 were a mixture of α form and amorphous form. MP2 had an amorphous form and MP 3 had a crystalline form β. The results of the solubility test showed that the β crystal form of rifaximin (MP3) had the lowest solubility. The amorphous form (MP2) had the highest solubility and the α-form and amorphous form mixtures (MP1 and MP4) had intermediate solubilities. In vitro comparative dissolution results showed that the dissolution profile of the leader brand medicine is not similar to that of the two generic brands G1 and G2 while G1 and G2 had similar comparative dissolution profiles. This was explained by the fact that the leader brand had a crystalline form of rifaximin (α-rifaximin) [1,11], while G1 contained the amorphous form or a mixture of α form and amorphous form (MP2, MP1) and G2 contained a mixture of α form and the amorphous form (MP4).

In this work, we have been able to highlight the impact of crystalline polymorphism of rifaximin on its solubility and dissolution, which were two key factors that have a definite impact on the bioavailability of this molecule. Our results were consistent with previous studies showing the influence of crystalline polymorphism of rifaximin on its bioavailability [1,11].

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Imen Toukabri: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Senda Bahri:** Visualization, Validation, Supervision, Resources. **Souad Sfar:** Validation, Supervision. **Mohamed Ali Lassoued:** Validation, Supervision, Resources, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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