

## **The impact of food components on the intrinsic dissolution rate of ketoconazole**

### Authors

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## **Abstract**

To accurately predict the *in vivo* performance of drugs from an *in vitro* dissolution test, the dissolution conditions used are supposed to be similar to those present in the gastrointestinal milieu. Post-prandial gastric fluid contains partially digested food mixtures consisting of fat, protein and carbohydrate. Despite this, the compendial dissolution medium recommended to simulate the gastric fluid is still composed of simple solution of hydrochloric acid and sodium chloride with or without the addition of pepsin. Therefore, in this investigation, biorelevant dissolution media were developed to evaluate the impact of food constituents; milk with different fat contents, egg albumin, gelatin, casein, gluten, carbohydrates and amino acids on the intrinsic dissolution behaviour of ketoconazole. Most of the food additives that were evaluated enhanced the apparent solubility of the drug but to different extents. The greatest enhancement in dissolution was observed in media containing either neutral amino acids or media based on milk mixtures. The formation of complexes between the drug and the additives most likely accounted for the solubilising effect and in milk-containing media the effect was attributed to the whole complex structure of milk rather than simply its fat content. These results highlight the potential effect of the type of ingested meal on drug dissolution and subsequent bioavailability.

## 1. Introduction

Dissolution testing is a potential predictive tool for the oral absorption of drugs but, to achieve this, test conditions need to simulate the physiological conditions prevailing in the intraluminal environment. Therefore, efforts have been made to develop biorelevant media which represent gastrointestinal fluids at fed and fasting states.

The composition of the gastric fluid in post-prandial conditions is variable according to the type of the administered meal <sup>(1)</sup>. Therefore, to tackle the problem of variability in gastric fluid composition, biorelevant dissolution media were suggested to standardize the composition of simulated gastric fluids at fed state. Milk was utilised as it is a nutrient that contains the three basic components of fat, protein and carbohydrates in a ratio resembling a typical diet <sup>(2)</sup>. In 2008, Jantratid et al. <sup>(3)</sup> recommended the use of a mixture of milk and acetate buffer and subsequently a formulation of milk digested with pepsin and lipase was proposed to represent the changes in intra-gastric composition according to the digestion stage <sup>(4)</sup>. Artificial liquid meals, such as Ensure™ and Intralipid™ were recommended as they are specifically designed to reflect the composition of a standard meal and can be well standardised <sup>(5)</sup>. The use of Ensure Plus™ containing a viscosity enhancer, 0.45% pectin <sup>(6)</sup> or medium containing 1.4% HPMC in acetate buffer <sup>(7)</sup> were suggested as an alternative media to the homogenised standard breakfast. A medium containing two types of vegetable oils, sucrose and surfactants was used to mimic fed gastric conditions <sup>(8)</sup>. Media based on mono-components of proteins, carbohydrates or amino acids were utilised as simple dissolution media that allowed the investigation of the effect of these individual components on the dissolution of drugs <sup>(9, 10)</sup>.

Drugs present in the gastric fluids are exposed to mixtures of foodstuffs made up of protein, carbohydrates, fats and amino acids and dissolution in the intra-gastric fluid is essential for

the subsequent absorption of basic lipophilic drugs, in particular. Hence, we previously evaluated the dissolution of itraconazole in biorelevant media <sup>(10)</sup>. Interestingly, the inclusion of food additives, such as milk and egg albumin, induced a pronounced increase in the dissolution of itraconazole, which suggested that the type of diet can influence drug dissolution *in vivo* and its subsequent bioavailability.

In the present investigation, these studies were extended to examine and compare the impact of similar biorelevant dissolution media on the dissolution behaviour of a second basic poorly soluble azole anti-fungal drug, ketoconazole. The drug is classified as class II according to the Biopharmaceutics Classification System (BCS) so it is more likely to exhibit dissolution-rate-limited absorption problems <sup>(2)</sup>. Clinical studies showed that when ketoconazole oral dosage forms were given with a breakfast meal the bioavailability of the drug increased <sup>(11)</sup>. The structure of ketoconazole (log P is 3.73) presents two basic groups, a piperazine ring and an imidazole ring, with pKa values of 2.94 and 6.51, respectively (Figure 1). Thus, there are two basic centres for protonation, N<sub>26</sub> of the piperazine ring and N<sub>11</sub> of the imidazole ring.

The solubility of ketoconazole has previously been studied in human gastric fluid <sup>(12, 13)</sup> and the dissolution of its oral dosage forms was investigated in biorelevant media based on milk <sup>(2)</sup>. However, studying the intrinsic dissolution of a drug through the determination of the intrinsic dissolution rate (IDR) from constant surface area of compact drug powder is of particular interest since it is independent of the formulation, excipient effects and the size of the particles <sup>(14)</sup>.

Thus, the objective of the present work was to characterise the intrinsic dissolution performance of ketoconazole in biorelevant dissolution media containing food constituents as a representative for gastric fluid at fed state.

## **2 Materials and methods**

### ***2.1 Chemicals and reagents***

Ketoconazole was obtained from Medichem (Shenzhen, Guangdong, China). Casein from bovine milk, gluten from wheat, gelatin from bovine skin, glycine (GLY), leucine (LYS), aspartic acid (ASP) were purchased from Sigma-Aldrich (Steinheim, Germany). Albumin from hen egg white, alanine (ALA) and lysine (LYS) were purchased from Fluka (Sigma-Aldrich, Steinheim, Germany). Glucose and starch were purchased from BDH (Poole, UK) and lactose was obtained from Foremost (Baraboo, Wisconsin, USA). Three types of fresh pasteurised bovine milk, whole milk, semi-skimmed milk and skimmed milk (Express dairies) with fat contents of 3.6%, 1.7% and 0.1%, respectively were purchased from local retail outlets (Liverpool, UK).

Sodium hydroxide pellets, potassium dihydrogen phosphate, sodium chloride and hydrochloric acid and as well acetonitrile were all purchased from BDH (Poole, UK). Triethylamine and n-butyl chloride were purchased from Fluka (Sigma-Aldrich, Steinheim, Germany).

### ***2.2 Composition of dissolution media***

Simulated gastric fluid (SGF) pH 1.2 without enzymes contained 2g/L NaCl and 7mL/L HCl (to adjust the pH to 1.2) in deionised water. SGF pH 3 also contained 2g/L NaCl in deionised water and the pH adjusted to 3 using 0.1M HCl.

The composition and preparation of the dietary media were as previously described <sup>(10)</sup>. SGF media containing milk were prepared using equal volumes of milk and SGF pH 1.2. The final pH was adjusted to 3 using either 0.1M HCl or 0.1M NaOH. Media containing a single dietary component were prepared by dissolving or dispersing the substance in SGF then the

final pH was adjusted to 3 with a solution of HCl (1 or 0.1M). Four proteins were chosen as models of proteins available in common diet. Albumin (0.01, 0.1, 0.5%, 1%, and 3%, w/v) and gelatin (1%, 2% and 4%, w/v) were directly added to SGF. A saturated solution of gluten was prepared by adding of an excess amount of gluten to deionised water and stirring overnight before filtration through Whatman filter paper type: 1 and the addition of NaCl (2g L<sup>-1</sup>). A filtered Casein solution was also prepared following same procedure and further dilutions (0.005, 0.0038, 0.0025 and 0.0013%, w/v) were prepared using SGF (pH 3). The final pH of all media was 3.

Amino acids were chosen to represent basic (LYS at 1%, w/v), neutral (GLY, ALA and LEU, each at 1%, w/v) and acidic amino acids (saturated solution of ASP). Three different carbohydrates were chosen as examples of monosaccharides (glucose), disaccharides (lactose) and polysaccharides (starch), each at 1%, w/v.

Simulated intestinal fluid (SIF) was prepared as described in the USP 32 <sup>(14)</sup>. Phosphate buffer pH (7.5) and acetate buffer (pH 5) were prepared according to the BP (2012) <sup>(15)</sup>.

### **2.3 Determination of saturation solubility**

The solubility of ketoconazole in each dissolution medium was determined using a modified ‘shake-flask method’ <sup>(16)</sup>. Each experiment was performed in triplicate. Medium (10mL) and approximately 300mg of drug powder were transferred into closed-cap vials, shaken gently for 24h at 37 °C in a shaking incubator (Model AM89B, Dynex Technologies Ltd). After this time, the suspension was centrifuged at 4000rpm for 10 min (Centaur 2, MSE, Fisons).

The supernatants were collected and filtered through a 0.2µm PVDF syringe with the first portion of each filtrate being discarded to circumvent any initial adsorption of the drug to the filters. Dissolution media which contained particulate matter (milk and albumin containing-

media) clogged these filters which required the use of filters with a bigger pore size <sup>(17)</sup>. Filters were validated prior to their use to verify that they did not adsorb the drug and hence affect the results of analysis. For the albumin-containing media, 1µm glass filters (Gelman Sciences) were used and 5µm Acrodisc Versapor filters (Gelman Sciences, medium: acrylic polymer) were used for the milk-containing samples. Ketoconazole filtrates were diluted as required with a mixture of acetonitrile-water (50:50, v/v) and then submitted to HPLC analysis.

#### ***2.4 Intrinsic dissolution disks and dissolution conditions***

Intrinsic dissolution rate (IDR) testing was performed using a stationary disk apparatus. A hardened polished steel plate covered with aluminium foil was attached to the steel die and the drug powder (approximately 200mg) was inserted into the cavity of the steel die (9.5mm diameter). The steel plate was occasionally re-polished to enhance the smoothness of the surface and so allow the dissolution to take place evenly across the surface <sup>(18)</sup>. A hardened-steel punch was inserted to die cavity then the whole arrangement was transferred to a hydraulic compressor and compressed under a pressure of 1000 p.s.i. for 30s yielding a disk of circular surface area. The disks were blown with compressed air to remove any loose particles.

Dissolution studies were carried out using USP apparatus II (paddle) (Pharmatest PTW S3C, Pharmatest GmbH). The die, containing the compressed drug powder, was positioned disk-up at the bottom of a flat-bottom dissolution vessel containing 500mL of the dissolution medium. The temperature was set at  $37 \pm 0.5^{\circ}\text{C}$  and a rotation speed of 100rpm was applied. Samples (3mL) were withdrawn periodically from the dissolution vessels through 0.45µm filters except for particulate complex dissolution media where samples were withdrawn through 20µm filters.

Compression is a high energy process which may affect the crystallinity of substances and different polymorphs may exhibit different solubility behaviours. Therefore, studies were carried out to determine whether compression of ketoconazole into IDR disks led to any change in polymorphic form. Differential scanning calorimetry, infra-red spectroscopy and powder x-ray diffractometry were employed and no evidence that compressing the ketoconazole powder affected the solid-state of the drug was observed.

### **2.5 *Extraction of ketoconazole from IDR sample solutions***

The drug was extracted from the complex dissolution media by liquid-liquid extraction at alkaline pH. Samples (1mL) removed from the dissolution media were alkalinised with 1mL of 0.05M NaOH and shaken mechanically for 10 min. 5mL of a mixture of acetonitrile:n-butyl chloride (1:4, v/v) was added, shaken for 1 min and then centrifuged at 4000 rpm for 10 min. 2mL aliquots were collected from the upper layer and evaporated to dryness with nitrogen at 60°C. The residues were reconstituted in 2mL of eluent (mobile phase used for HPLC) by sonication for 2 min and subsequently analysed using HPLC.

### **2.6 *Analytical method***

Drug quantitation was carried out using a HPLC separation module Waters Alliance 2695 chromatograph using a Waters 996 Photodiode Array Detector (PAD). Ketoconazole samples (20µL) were eluted using a mobile phase consisting of acetonitrile-water-triethylamine (50:50:0.1 v/v/v), with a flow rate of 1.1mL min<sup>-1</sup>. A 5µm Hypersil BDS C18 column (150 mm x 4.6mm) (Thermo Electron Corporation) fitted with a Phenomenex C18 guard cartridge (4 mm x 3mm) was used and eluting peaks were detected at a wavelength of 254nm. Quantitation was based on the use of an external ketoconazole calibration standard and peak area measurement. A plot of the amount dissolved per unit area versus time was constructed



and the slope of the fitted linear regression represented the IDR. The initial dissolution rate referred to the period 0-15min and the subsequent rate referred to the period 15-240min.

### **3 Results and discussion**

#### ***3.1 Dissolution and saturation solubility in conventional media***

Ketoconazole solubility was highly dependent on the pH of the medium. At pH 3 all the imidazole moieties ( $pK_{a1}=6.5$ ) and nearly 50% of the piperazine moieties ( $pK_{a2}= 2.9$ ) were protonated. By lowering the pH further to 1.2, both base moieties were protonated leading to a significant increase in solubility under the more acidic conditions (Table 1).

The IDR of the drug could not be determined at pH 1.2 due to its high solubility at that pH whereby the disks entirely dissolved within 15min. The IDR data at pH 3 and at pH 6.8 presented in Table 2 indicate the profound effect of the pH of the dissolution medium on the rate and extent of drug dissolution. The dissolution data in SGF (pH 3) were considered as a reference and employed for subsequent comparison with the dissolution profiles in more complex media.

The increase in the dissolution rate and solubility at pH 1.2 confirms the importance of the gastric acidity on the bioavailability of ketoconazole. Clinical studies reported that the bioavailability of the drug decreased when the gastric pH increased by administering the drug concomitantly with antacids or H<sub>2</sub> blockers <sup>(19)</sup>.

#### ***3.2 Dissolution and saturation solubility in milk-containing media***

The solubility of ketoconazole in milk-SGF mixtures (1:1) was approximately 6 times greater than the solubility in SGF alone (Table 3). No significant difference between the solubility

values for the three different milk-containing media was observed, according to the one-way analysis of variance (ANOVA) at significance level of 0.05.

The initial IDR in whole fat milk-containing medium was 1.3-fold greater than that in SGF and was slightly higher than the values obtained for the other two milk-containing media (Figure 2). A greater increase was observed in the IDR of the subsequent time period (15-240min), of around 5.4-fold compared to SGF. No differences were apparent in the amount dissolved in the three milk based media during the first 180min and after that the dissolution in skimmed milk-containing media was lower.

Ketoconazole is a lipophilic molecule, and therefore the increased fat content of milk would be expected to aid solubility, but the different fat content of the three types of milk did not show the expected systematic effect on drug behaviour. The same observations were noted previously with itraconazole where the different fat content of milk-containing media did not affect the behaviour of itraconazole in the expected systematic manner <sup>(10)</sup>. It is possible that some disruption of the milk emulsion occurred during the dissolution experiment which led to separation of the fat, which would then float to the surface <sup>(20)</sup>, making it unavailable to the drug compact at the bottom of the vessel. This is probably due to the destabilizing effect of adding acid to milk. Another possibility is the presence of the milk fat in separate small globules coated with a membrane which acts as a barrier between the fat and the milk plasma and prevents the globules from coalescing <sup>(21)</sup>. Thus, the composition of the membrane may physically hinder the fat solubilising effect on the lipophilic drugs.

Thus, a possible mechanism by which milk enhanced the dissolution rate of the drug could be via a solubilising effect of the complex structure of milk and its ability to bind drugs <sup>(22)</sup>.

### 3.3 *Dissolution and saturation solubility in the presence of proteins*

The solubility of ketoconazole in albumin-containing media clearly increases in the presence of albumin (Figure 3). A strong correlation was observed between the concentration of albumin and ketoconazole solubility up to 1%, w/v of albumin.

An increase in drug dissolution was also apparent in media containing albumin (Figure 4).. However, there was a decline in the initial IDR with the inclusion of low concentrations of albumin, compared to SGF, whereas a slight increase was observed with higher concentrations of albumin (1 and 3 %, w/v).

Egg albumin induced an interesting solubilising effect which was proportional to the concentration of the protein. This effect is attributed to interactions between the protein and the drug through hydrophobic, electrostatic interactions and hydrogen bonding. The possible sites of hydrophobic interactions in the drug molecules are the aromatic ring and the 2, 4-dichloro-phenyl ring whilst possible multi-hydrogen bonding sites include the unionised nitrogens and the carbonyl groups adjacent to piperazine ring. This is similar to the case seen with human serum albumin where ketoconazole exhibited high protein binding *in vivo*, the mechanisms of the interaction were electrostatic interaction and hydrophobic interactions <sup>(23)</sup>.

The increase in the dissolution rate was not to the same extent as the increase in solubility. For instance, the solubility increased 3-fold in 1%, w/v albumin-containing medium compared to SGF but the initial dissolution rate of the drug in the same medium only increased by a factor of 1.2 but at the subsequent step (15-240min) it increased by a factor of 2.6. The relatively slow dissolution during the first step could be ascribed to the formation of larger complexes which made their diffusion to the bulk solution slower than the free drug <sup>(9)</sup>.

The solubility studies indicated a slight enhancement in solubility in 0.0038 and 0.005%, w/v casein-containing solutions whereas the more dilute solutions of casein did not induce a significant effect (Table 3). The data presented in (Figure 5) demonstrate that there was an increase in dissolution rate of ketoconazole in casein saturated solution compared to the SGF. The initial IDR was 1.5-fold greater than its counterpart in SGF however the IDR representing the subsequent stage in casein solution was not determined due to its poor linearity.

It was reported previously that the mechanism by which casein affected drug dissolution was via micelle formation <sup>(9)</sup>. Casein molecules have a strong tendency to self-assemble into micelles because of their amphiphilic nature in aqueous solution. Various models of the assembly and structure of the casein have been suggested in the literature of which the sub-unit model for casein aggregation was the most widely accepted. This model suggests that 15–20 molecules of casein aggregate via hydrophobic interactions and form sub-units where the hydrophobic core is surrounded by a polar portion. These sub-units form the building units of the micelles <sup>(24)</sup>. Casein monomers and sub-units exist in the solution when the casein concentration is below the CMC, whereas above the CMC casein micelles are combined with monomers and sub-micelles. Based on this structure, casein is able to encapsulate hydrophobic compounds into the hydrophobic core even at the sub-micellar level. Consequently, ketoconazole could be solubilised through these sub-units.

The inclusion of gluten in the media did not induce a discernible effect on either the solubility or the dissolution rate of ketoconazole (Figure 5). This indicated that this protein could not solubilise ketoconazole which agrees with the previous finding regarding itraconazole dissolution in this medium <sup>(10)</sup>.

Both parameters; solubility and IDR, increased significantly in the presence of gelatin in SGF. The solubility increased linearly with gelatin concentration compared to SGF (Table 3). The gelatin-containing media induced similar initial dissolution patterns; however within 3h there was clear evidence that the increase in the dissolution rate was proportional to gelatin concentration (Figure 6). The increase in the dissolution rate and the solubility was approximately equivalent in these media.

The effect of gelatin may be attributed to its surface activity <sup>(25)</sup> where gelatin-containing solutions have been seen to enhance the wettability of the drug compact surfaces.

### ***3.4 Dissolution and saturation solubility in the presence of amino acids***

The inclusion of 1%, w/v GLY in the medium increased the solubility 11-fold whereas LYS increased it only 6-fold. ASP did not induce a discernible effect. Consequently, the effect of further neutral amino acids (ALA and LEU) was investigated. Each neutral amino acid-containing media showed a considerable solubility enhancement of ketoconazole (Figure 7). A parallel increase in the dissolution rate of ketoconazole was observed in these media. The dissolution test was performed for 180min in the amino acid-containing media as holes formed at the surface of the disks after 3h. Figure 8 shows that the initial IDR was greater in solutions containing amino acids than in SGF but there was no significant difference between the various amino acid media. However, from 15 minutes onward clear differences were seen.

This comparison in solubility was based on media which each contained the amino acid at a level of 1% w/v. In terms of molarity a 1%, w/v GLY solution is 133mM so equimolar solutions of LYS (1.9%, w/v) and ALA (1.2%, w/v) were prepared. It was found that ALA induced the greatest enhancement in solubility of approximately 11.6-fold whereas the increase in LYS-containing media was 7.8-fold.

Thus, it could be said that the inclusion of neutral amino acids induced a significant increase in dissolution and solubility. Since these amino acids have identical isoelectric points ( $PI=6$ ); the greatest effect being seen with LEU- then ALA- and finally GLY-containing media, suggest the increase in solubility and dissolution was possibly proportional to the length of side chain of the amino acids and consequently their overall hydrophobicity.

This effect of the amino acids could be attributed to the formation of a soluble complex with the drug. At pH 3, ketoconazole molecules coexist in the two ionised forms: mono-protonated and di-protonated. The acidic groups of the amino acids have a  $pK_{a1}$  of 2.3 and thus at pH 3, the majority of these acidic moieties are deprotonated. Consequently, ionic interactions may occur between the negatively charged carboxylic acid of the amino acid and the positively charged drug molecules. Furthermore, hydrogen bonding may also contribute to the interactions where the uncharged nitrogen or carbonyl groups of the drug are possible sites for hydrogen bonds with the carboxylic acid groups of the amino acid. In addition, the solubility of ketoconazole increased with an increase in the hydrophobic character of the amino acid in the dissolution medium suggesting that hydrophobic interactions could be an important force in the interactions.

To evaluate the effect of the pH of the medium on the amino acid-solubilising effect, dissolution was further investigated in SIF (pH 6.8) containing 1%, w/v GLY. In this instance GLY only increased the dissolution rate 1.8-fold and the solubility 1.3-fold, compared to SIF. This indicated that the solubilising effect of GLY at pH 6.8 (SIF) was clearly less than at pH 3 (SGF), presumably because ketoconazole has lost most of its positive charge at this pH and is therefore less able to complex with the amino acid anions.

### ***3.5 Dissolution and saturation solubility in the presence of carbohydrate***

Including glucose and starch in the medium did not affect ketoconazole solubility or dissolution rate whereas a slight increase in dissolution rate was seen with lactose-containing medium (Figure 9). Although carbohydrates can also form complexes via hydrogen bonding between hydroxyl groups in sugars and the nitrogen or carbonyl groups in drug entities, this does not appear to have occurred to any great extent in the case of ketoconazole.

### ***3.6 Comparison of the dissolution of Ketoconazole and itraconazole in bio-relevant media***

As previously mentioned, the intrinsic dissolution of itraconazole was evaluated in a variety of biorelevant media <sup>(10)</sup>. Thus, comparing the data acquired from ketoconazole and from itraconazole; in general, it was observed that food additives induced the same positive effects on both drugs; however the extent of the effect varied. For instance, the effect of egg albumin (1%, w/v) was more pronounced with itraconazole than with ketoconazole whereas the inclusion of amino acids induced a greater solubilising effect on ketoconazole than itraconazole.

Although both drugs are BCS Class II and have azole structures, differences were observed in their solubility and dissolution behaviours. The different physiochemical properties of both drugs, in particular, pKa and log P had an important impact on their behaviour. Itraconazole (pKa= 3.7 and log P=5.66) is more lipophilic, has extremely poor aqueous solubility ( $\sim 0.002 \mu\text{g mL}^{-1}$ ) and is mono-ionic at pH 3 whereas ketoconazole is relatively more water-soluble ( $5.4 \mu\text{g mL}^{-1}$ ) and more positively charged at the same pH. Accordingly, these variations led to different solubilities and interactions with the additives and consequently different dissolution patterns of the two drugs.

## 4 Conclusions

Although food has a buffering effect on the acidity of the gastric fluid which in turn is thought to suppress the bioavailability of the two basic drugs; itraconazole and ketoconazole, clinically an increase in the bioavailability of the drugs was reported. In the present study it was observed that the dissolution of ketoconazole increased with the presence of most of the dietary additives investigated. Consequently, increased drug dissolution could be identified as a reason for the increasing bioavailability of this poorly soluble drug at the post-prandial state, in addition to the physiological changes in response to food ingestion such as promotion in the bile secretion and extending the gastric residence time. Since additives such as milk, egg albumin and gelatin increased the solubility of the drug, it might be useful to employ these substances in developing drug formulations as the use of such natural vehicles in appropriately designed formulations may enhance the solubility of the poorly soluble drugs and provide safe and cheap alternative for the synthetic polymers.

In conclusion, biorelevant media based on food constituents are recommended for the prediction of the behaviour of the drugs in the gastrointestinal tract as they appear as more realistically representative of the intraluminal fluids than the simple compendial dissolution media. Such media have the potential to reflect changes in the performance of BCS Class II drugs due to fed and fasting conditions and, even more specifically, due to the type of the meal which in turn can affect drug bioavailability. Clinicians can benefit from such information when prescribing drugs to achieve the desired bioavailability and therapeutic efficacy.



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**Declarations of Interest**

The authors report no declarations of interest.

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**Table 1 Solubility of Ketoconazole in SGF, SIF, acetate buffer, phosphate buffer and deionised water. Each data point represents the mean  $\pm$  SD of 3 measurements**

<b>Medium</b>	<b>Ketoconazole Solubility (mg mL<sup>-1</sup>)</b>
SGF (pH 1.2)	20.33 $\pm$ 3.26
SGF (pH 3)	0.43 $\pm$ 0.05
Acetate buffer (pH 5)	0.1 $\pm$ 0.009
SIF (pH 6.8)	0.007 $\pm$ 0.001
Phosphate buffer (pH 7.5)	0.006 $\pm$ 0.001
Deionised water	0.0054 $\pm$ 0.0005

**Table 2 Intrinsic dissolution rate (IDR) data of ketoconazole in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and in SGF containing milk or proteins (albumin, gelatin, casein or gluten). Each data point represents the mean  $\pm$  S.D. of 3 measurements**

<b>Medium</b>	<b>Initial IDR (0-15min) (<math>\mu\text{g min}^{-1} \text{cm}^{-2}</math>)</b>	<b>Subsequent IDR (15-240min) (<math>\mu\text{g min}^{-1} \text{cm}^{-2}</math>)</b>
SGF pH 3	187.3 $\pm$ 27.3	75.4 $\pm$ 3.5
SIF pH 6.8	43.4 $\pm$ 7.9	1.4 $\pm$ 0.1
SGF-Whole fat milk	247.7 $\pm$ 40.5	407.7 $\pm$ 37.5
SGF-Semi-skimmed milk	242.7 $\pm$ 39.8	433.7 $\pm$ 58.5
SGF-Skimmed milk	206.4 $\pm$ 13.7	311.3 $\pm$ 6.8
Albumin 1%	217.6 $\pm$ 6.3	194.8 $\pm$ 9.3
Casein filtered solution	284.0 $\pm$ 45.4	-
Gluten filtered solution	207.0 $\pm$ 11.4	71.6 $\pm$ 13.1
Gelatin 1%	332.0 $\pm$ 47.8	250.8 $\pm$ 8.8

**Table 3 Solubility of ketoconazole in SGF, SGF-milk mixtures (semi-skimmed, skimmed, whole fat) and in SGF containing different concentrations of casein, gelatin and gluten. Each data point represents the mean  $\pm$  S.D. of 3 measurements**

<b>Medium</b>	<b>Ketoconazole Solubility (mg mL<sup>-1</sup>)</b>
Casein 0.0013%	0.404 $\pm$ 0.027
Casein 0.0025%	0.403 $\pm$ 0.007
Casein 0.0038%	0.509 $\pm$ 0.015
Casein 0.005%	0.533 $\pm$ 0.008
Gluten solution	0.414 $\pm$ 0.040
Gelatin 0.5%	0.80 $\pm$ 0.04
Gelatin 1%	1.08 $\pm$ 0.02
Gelatin 2%	1.50 $\pm$ 0.08
SGF-Semi-skimmed milk	2.30 $\pm$ 0.21
SGF- skimmed milk	2.53 $\pm$ 0.15
SGF-Whole fat milk	2.66 $\pm$ 0.36

## Figure Captions

Figure 1 Chemical structure of ketoconazole

Figure 2 The intrinsic dissolution profile of ketoconazole in simulated gastric fluid (SGF) (■) and in SGF-milk mixtures (1:1) (whole fat (Δ) semi-skimmed (●) or skimmed (◆). Each data point represents the mean  $\pm$  S.D. of 3 measurements

Figure 3 The effect of egg albumin concentration on the solubility of ketoconazole

Figure 4 The intrinsic dissolution profile of ketoconazole in simulated gastric fluid (SGF) (■) and in SGF containing egg albumin in concentrations of 0.01% (▽), 0.1% (▲), 0.5% (□), 1% (▼) and 3% (○), w/v. Each data point represents the mean  $\pm$  S.D. of 3 measurements

Figure 5 The Intrinsic dissolution profile of ketoconazole in simulated gastric fluid (SGF) (■) and in SGF containing casein (▽) or gluten (▲) filtered solutions. Each data point represents the mean of 3 measurements  $\pm$  S. D.

Figure 6 The intrinsic dissolution profile of ketoconazole in simulated gastric fluid (SGF) (■) and in SGF containing gelatin in concentrations of 1%(●), 2%(Δ) and 4%(▼), w/v. Each data point represents the mean  $\pm$  S. D. of 3 measurements

Figure 7 Solubility and IDR of ketoconazole in simulated gastric fluid (SGF) and SGF containing amino acids. Each data point represents the mean  $\pm$  S.D. of 3 measurements



Figure 8 The intrinsic dissolution profile of ketoconazole in simulated gastric fluid (SGF) (■) containing 1%, w/v amino acids LYS(▽), GLY(▲), ALA(○) and LEU(◆). Each data point represents the mean  $\pm$  S.D. of 3 measurements

Figure 9 The intrinsic dissolution profile of ketoconazole in SGF (■) and SGF containing 1% carbohydrates (glucose (▲), lactose (Δ) and starch (▽)). Each data point represents the mean  $\pm$  S.D. of 3 measurements

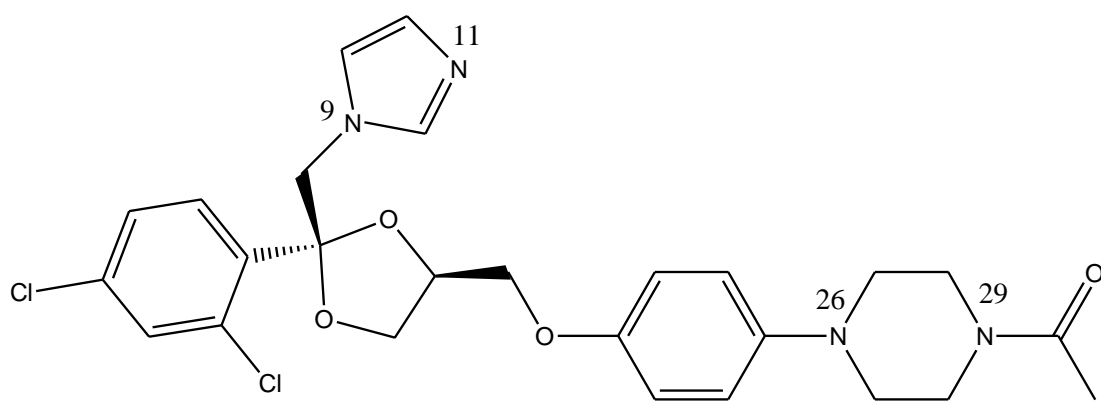


Figure 1

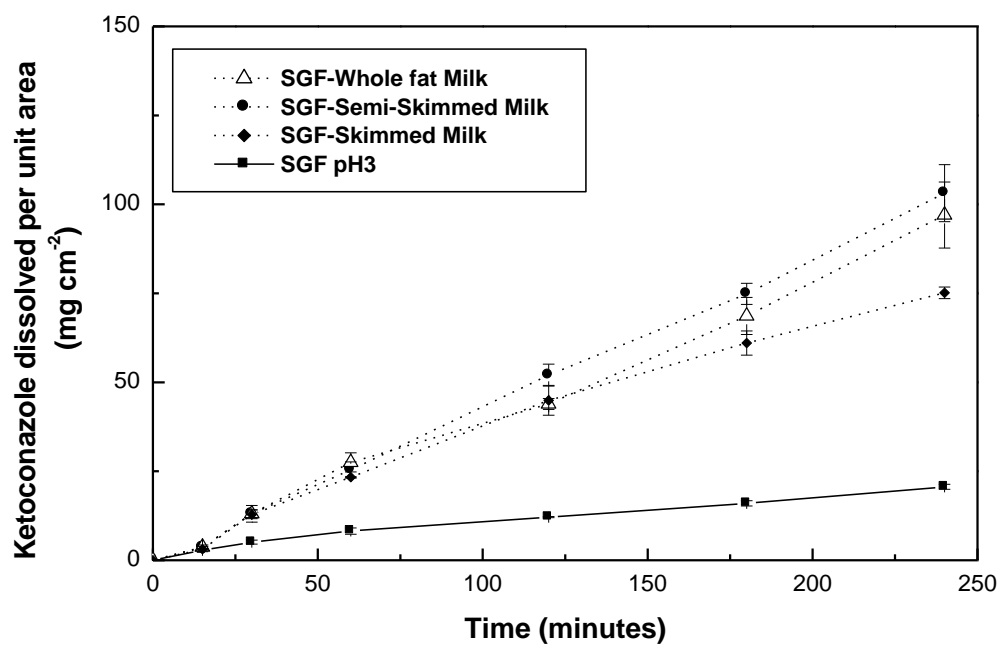


Figure 2

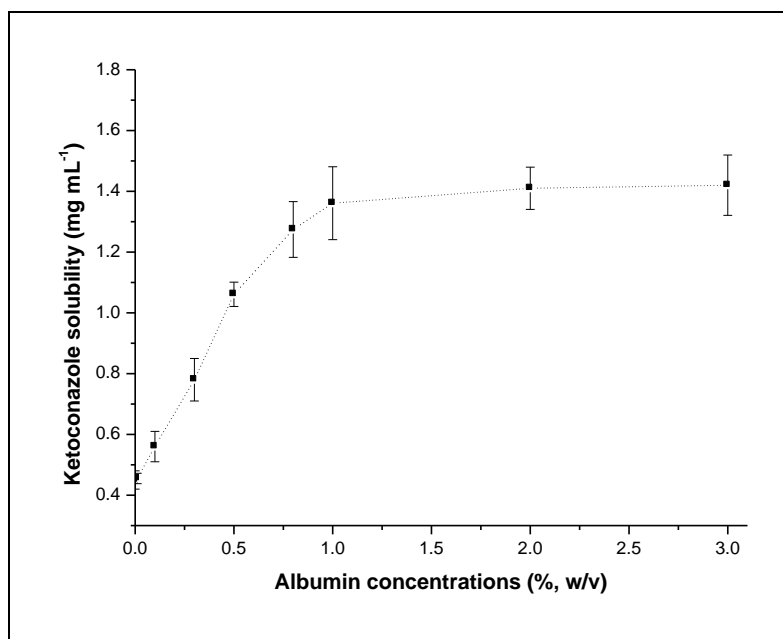


Figure 3

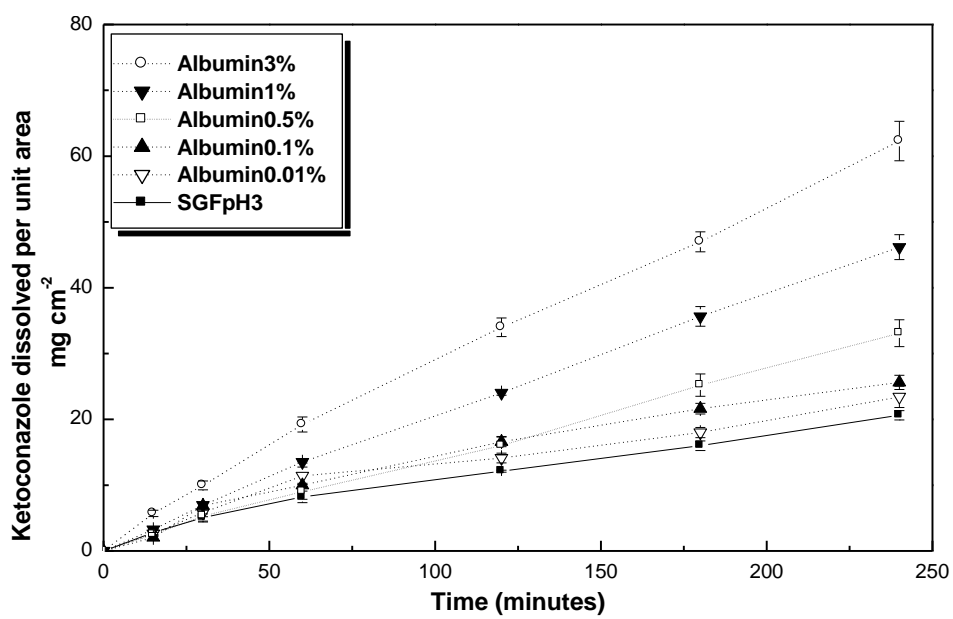


Figure 4

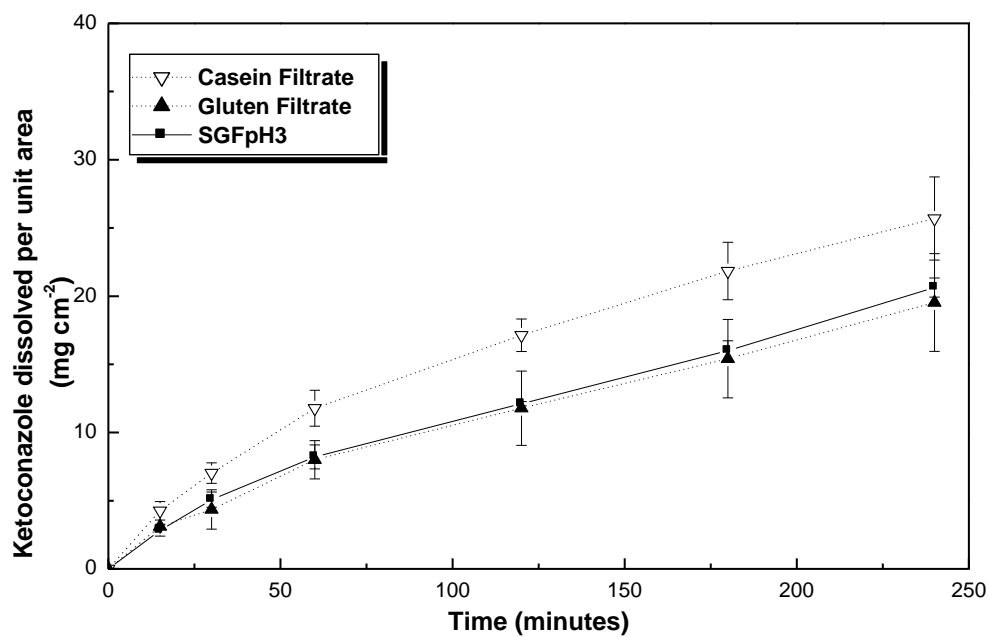


Figure 5

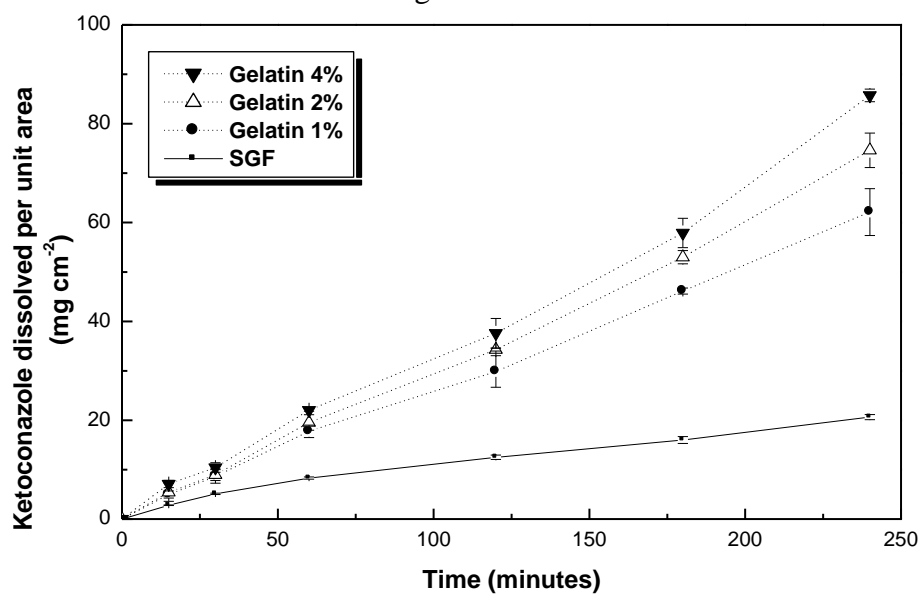


Figure 6

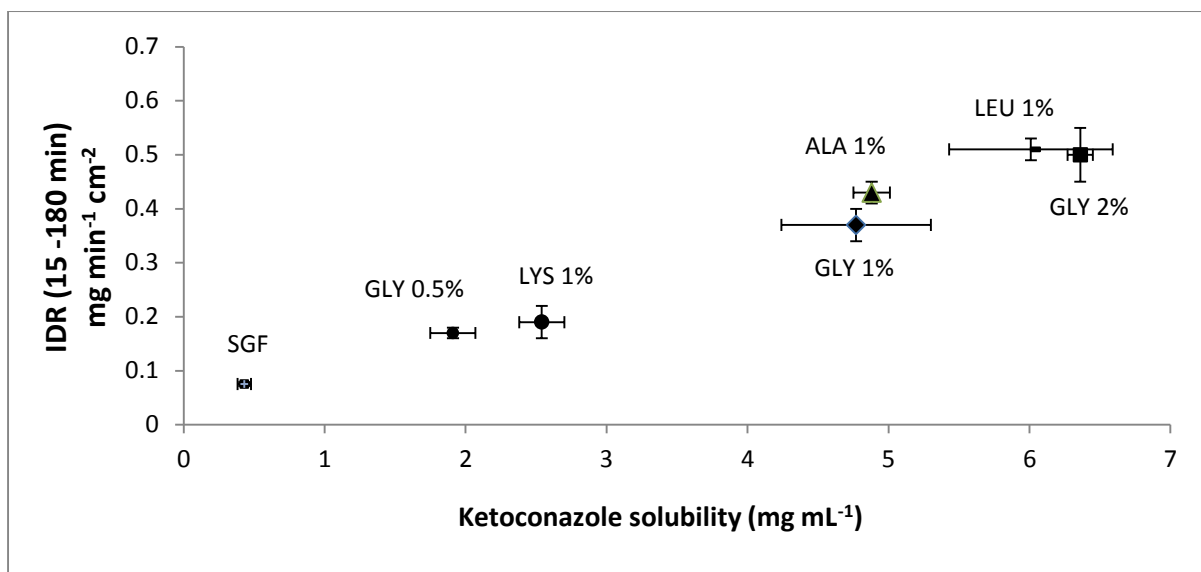


Figure 7

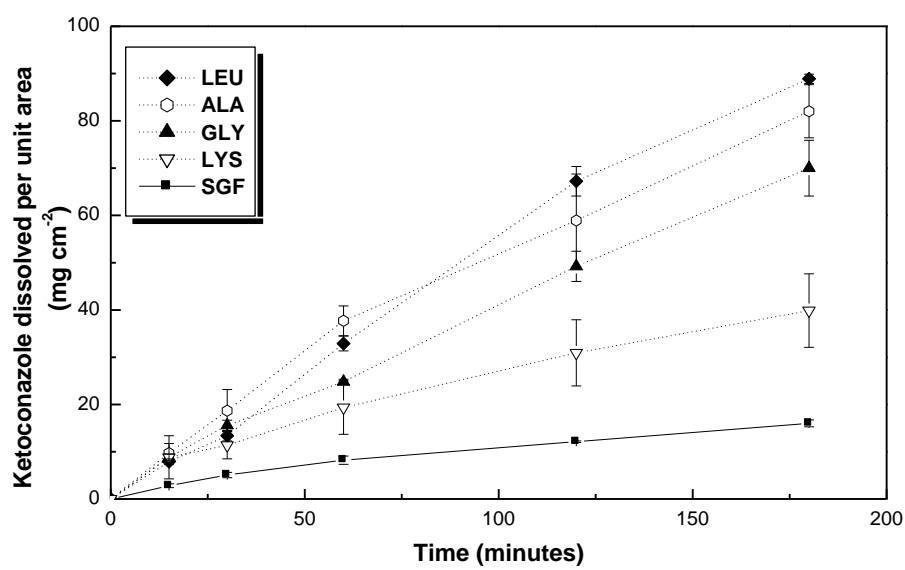


Figure 8

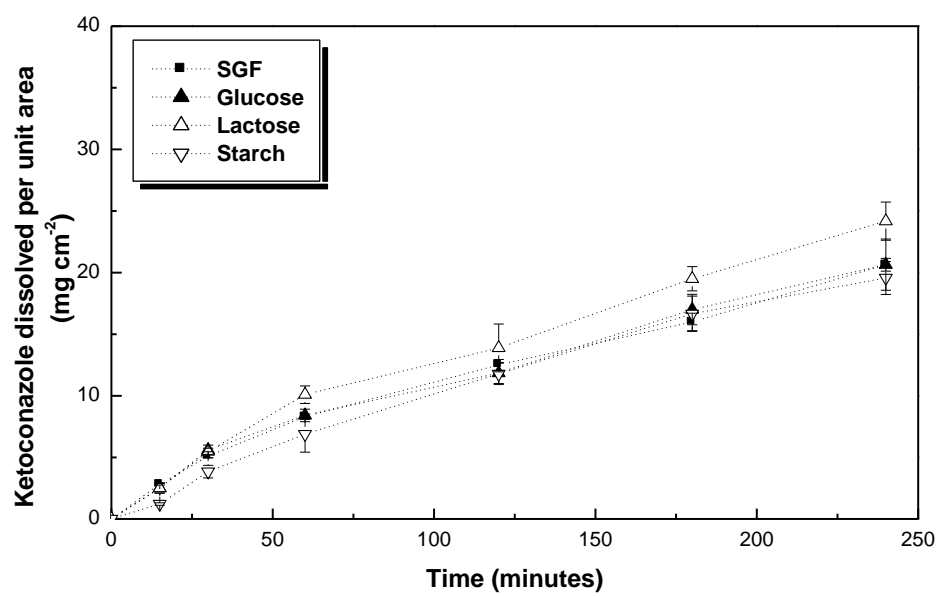


Figure 9

