

THE LACK OF BIOLOGICAL RELEVANCE OF THE OFFICIAL *IN VITRO* DISSOLUTION METHODOLOGY FOR THE IMMEDIATE RELEASE SOLID ORAL DOSAGE FORMS OF KETOCONAZOLE

MITU MIRELA ADRIANA¹, RĂDULESCU FLAVIAN ȘTEFAN^{2*},
HÎRJĂU MIRCEA¹, LUPULEASA DUMITRU¹, DUMITRESCU ION-
BOGDAN³, MIRON DALIA SIMONA³

*University of Medicine and Pharmacy „Carol Davila” Bucharest,
Faculty of Pharmacy, 6th Traian Vuia street, 020956, Bucharest,
Romania.*

¹*Department of Pharmaceutical Technology and Biopharmaceutics,*

²*Department of Drug Industry and Pharmaceutical Biotechnologies,*

³*Department of Pharmaceutical Physics and Informatics*

**corresponding author: flavian_stefan@yahoo.com*

Abstract

The paper presents the effect of drastic pH variations during *in vitro* drug release tests on the dissolution profiles of ketoconazole reference immediate release oral solid dosage form. The acidic media induced a rapid and almost complete dissolution of ketoconazole, while the pH change determined an accelerated and continuous precipitation process, almost not dependent on the stirring rate. The results indicate that for generic drug products containing lipophilic weak bases, a rapid dissolution compared to the reference product could explain the reduced biopharmaceutical performances.

Rezumat

Lucrarea prezintă efectele variațiilor drastice de pH induse pe durata testelor de cedare *in vitro* asupra profilelor de dizolvare ale ketoconazolului din forma farmaceutică solidă orală de referință. Mediu acid a indus o dizolvare rapidă și aproape completă a ketoconazolului, iar schimbarea de pH a determinat un proces de precipitare accelerată și continuă, aproape independentă de viteza de agitare. Rezultatele indică faptul că pentru produsele generice conținând compuși slab bazici lipofili, dizolvarea rapidă comparativ cu produsul referință poate explica performanțele biofarmaceutice reduse.

Keywords: ketoconazole, tablets, dissolution test, media change.

Introduction

The development of generic solid oral dosage forms containing antifungal azoles with immediate release profiles frequently generates lower *in vivo* performance, compared to the reference product. This experimental fact is explained by the acido-basic properties of the drug molecule, especially the weak base properties of ketoconazole [1].

The dissolution tests performed for formulation screening during the development phase of a generic drug product use a medium that assures the accurate solubility of ketoconazole (0.1N hydrochloric acid solution, pH=1.2), without considering the influence of the typical gastro-intestinal pH gradient on the solubility [2]. This gradient is correlated with the generation of oversaturated solution in some instances. The low value of the solubility at the site of absorption (intestinal segment), although associated with a fast dissolution in the gastric environment, induces a reduced bioavailability and *in vitro* – *in vivo* non-correlations [3].

The current paper evaluated the impact of pH variation on the dissolution profile of ketoconazole from immediate release oral solid dosage forms. Also, the results generated according to two available dissolution monographs were compared (United States Pharmacopeia 30 – National Formulary 25 (USP30-NF25, [4]) recommended procedure and a test with media change, designed for the evaluation of modified release oral drug products). The evaluation of the results was performed based on the physico-chemical properties of ketoconazole, but also on the physiological parameters with considerable influence on its biopharmaceutical profile (pH, motility etc.) [5].

Materials and Methods

Two sets of *in vitro* dissolution studies were conducted using two USP 2 dissolution apparatus: Vankel 7000, Vankel Technology Group, USA, and Esadissolver 3, Advanced Products SRL, Italy.

The first test was performed on six units of drug product (NizoralTM tablets, SC Terapia SA, in collaboration with Janssen Pharmaceutica N.V. Bersee, batch no. 02050143). The dissolution media was composed of 900mL solution of hydrochloric acid 0.1N (pH=1.2), according to the USP30-NF25 [4] recommendations. Samples of 1.0 mL were collected at 5, 10, 15, 20, 30, 45 and 60 minutes after the debut of the test.

The second experimental procedure represented an adaption of a typical, modified release drug product test recommendation: the initial use of an acidic dissolution media [4] (750 mL solution of hydrochloric acid 0.1N (pH=1.2), followed by a rapid pH change induced by adding 250 mL of 2M trisodium phosphate solution. The final pH of the media was 7.2, with a concentration of the buffer system of 50 mM. Five domains of agitation rate were implemented (50, 55, 60, 65 and 70 rpm), with probes of 1.0 mL sampled at 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 105 and 120 min. These tests were performed on three units of drug product, at 37°C.

The dissolution media degassed by filtration, using cellulose acetate filters (46 mm diameter, 0.45 μm pore diameter, with an Agilent Technologies filtration system), the filtrate being maintained at 41°C under 900 mBar vacuum. The pH of the dissolution media was controlled using a Combo HI 98130, Hanna Instruments pH-meter, before distribution into the dissolution vessels.

The sampled probes were filtrated using canula filters immersed in the dissolution media (10 μm pore size).

The quantitative evaluation of dissolved ketoconazole amounts was performed using a spectrophotometric method (at 221 nm), after adequate dilution with the corresponding media (either acidic or phosphate buffer) and further interpolation on the calibration curves (using a double fascicle, Jasco V-530 model spectrophotometer).

All the other reagents and the analytical standard of ketoconazole were purchased from Sigma Aldrich. The purified water was generated by a SGW Ultraclear UV PlusTM system.

Results and Discussion

Two types of dissolution methodologies were implemented for ketoconazole immediate release oral solid dosage forms. The first test confirmed the rapid and almost complete dissolution of the active pharmaceutical ingredient in the compendial recommended acidic media (figure 1). This release pattern is typical for the compounds with weak base characteristics, determining an accurate solubility within the gastric environment.

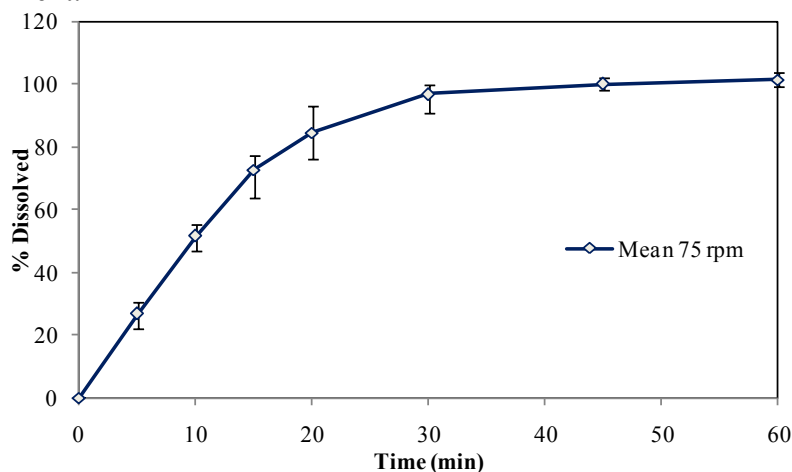


Figure 1

Mean dissolution profile of ketoconazole tablets at 75 rpm in acidic media (N=6)

The second set of tests represented an adaption of the compendial monograph for evaluation of the media change on the *in vitro* drug release profile of the modified release oral solid dosage forms. The final pH of the dissolution media, induced by adding the 50 mM trisodium phosphate 2 M solution, corresponded to the typical values of the physiological environment, that is the jejunum-ileum segment, the major site of absorption by passive diffusion processes, with or without the involvement of the active transport systems.

The five different values of agitation rate, in the interval of 50 to 70 rpm, in steps of 5 rpm, intended to investigate, within the pH dependence of the solubility profile, the influence of the hydrodynamics on the amount dissolved. This last parameter could be associated to the inter-individual variability of the gastro-intestinal motility.

During the first 30 minutes (corresponding to the half-life of the gastric evacuation rate) the amount of ketoconazole dissolved was directly correlated to the agitation rate, the differences being considered as not significant (the profiles were similar, with 95.26% dose dissolved for 50 rpm, and 98.11% for 70 rpm, respectively) (figure 2).

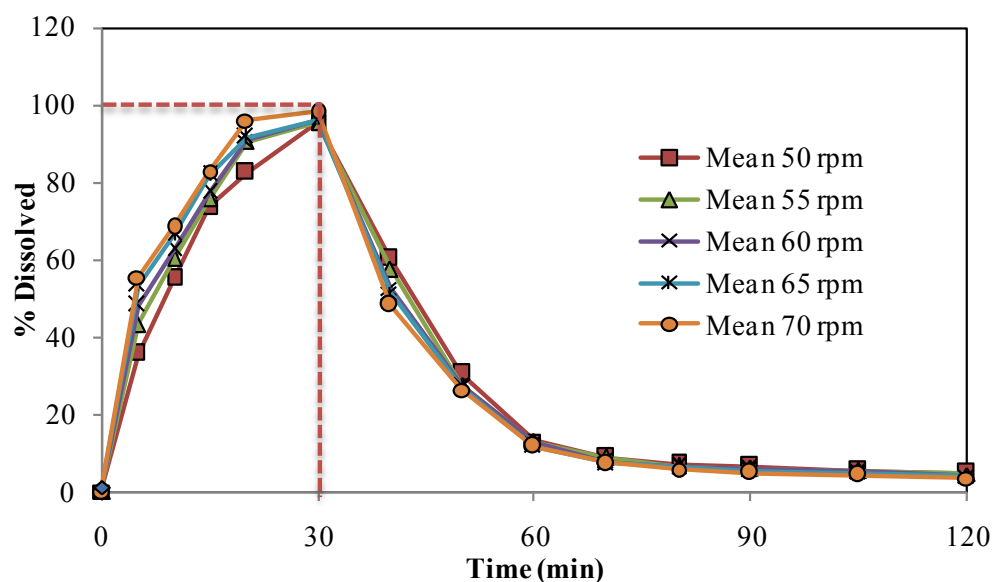


Figure 2

Mean dissolution profiles of ketoconazole tablets at 50, 55, 60, 65 and 70 rpm (media change after 30 minutes; N=3)

The reported differences between the two types of *in vitro* drug release tests at 30 minutes (e.g. the higher amount dissolved of ketoconazole at 60, 65 and 70 rpm in 750 mL, compared to the 75 rpm in 900 mL acidic environment, with values of similarity factor lower than 50) could be explained partially by the assumed, existing differences in hydrodynamic parameters induced by the volume of dissolution media.

Table I

The influence of dissolution test conditions on the similarity factor, f_2 [5]

a) time interval: 0-30 minutes.

f_2	75 rpm	50 rpm	55 rpm	60 rpm	65 rpm	70 rpm
75 rpm	100	69.27	54.85	49.6	44.03	41.55
50 rpm	69.27	100	64.39	57.44	49.98	46.24
55 rpm	54.85	64.39	100	80.04	62.25	57.23
60 rpm	49.6	57.44	80.04	100	73.29	64.78
65 rpm	44.03	49.98	62.25	73.29	100	79.99
70 rpm	41.55	46.24	57.23	64.78	79.99	100

b) time interval: 0-120 minutes.

f_2	50 rpm	55 rpm	60 rpm	65 rpm	70 rpm
50 rpm	100	71.92	64.05	57.42	53.3
55 rpm	71.92	100	82.2	69.31	63.28
60 rpm	64.05	82.2	100	80.98	71.94
65 rpm	57.42	69.31	80.98	100	84.47
70 rpm	53.3	63.28	71.94	84.47	100

The instant variation of pH value generated an intense precipitation of ketoconazole, process that continued during the next 90 minutes. It is to be mentioned the impact on the remaining amount dissolved of ketoconazole, inversely correlated with the quantity released at the moment when the pH change was induced (4.61% for 50 rpm and 3.39% for 70 rpm) (figure 3).

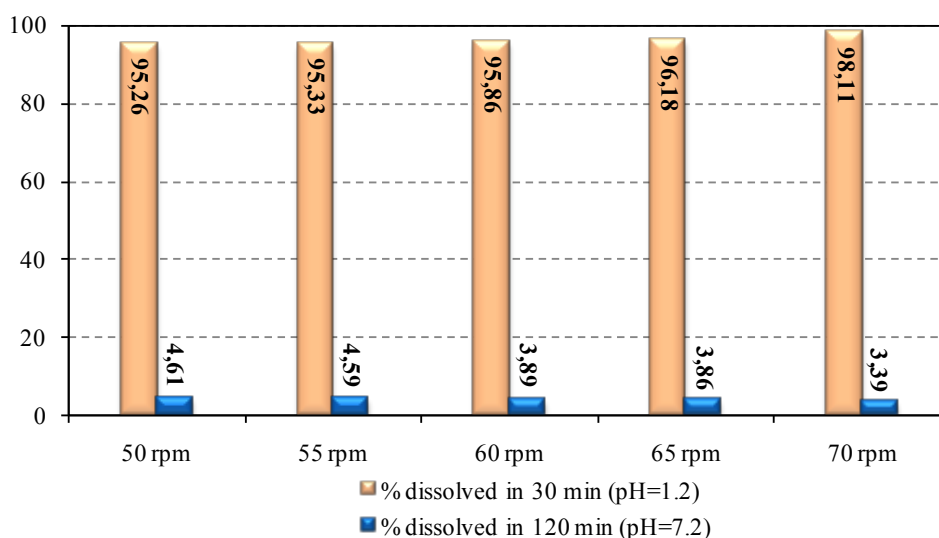


Figure 3

The variation of the ketoconazole amount dissolved at 30 and 120 minutes

The similarity of the “precipitation profiles” could be explained, but the final volume of the *in vitro* drug release media has no physiological correspondence [5,6]. At the gut level, the presence of small volumes (approximately 100-200 mL of intestinal fluid), with regional distribution, could drive to a more intense precipitation, even in the presence of endogenous tensioactive agents, such as bile acids and salts [7].

Conclusions

For basic drug substances, administered as immediate release solid oral dosage forms, a fast dissolution within the gastric environment could be followed by an intense precipitation in the gut lumen, generating a slow, solubility limited absorption process.

It is the paradox of inferior bioavailability reported by the drug manufacturers when the generic formulation releases faster compared to the reference one, since compendial dissolution test results are assumed to display also a biological relevance.

The currently recommended drug dissolution tests are intended for batch quality control purposes (within Scale Up Post Approval Changes framework), in order to reveal the impact of various formulation and/or process changes.

One of the approaches available in order to avoid the non-biosimilarity of drug products containing basic active pharmaceutical

ingredients could be a different acceptance criteria for the similarity factor, while requesting similar release kinetics and a lower mean difference (<10%).

Acknowledgements

This paper was partially supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64153.

References

1. Mircioiu C, Voicu V, Miron D, Mircioiu I: Non-standard correlations: in vitro in vivo correlations for immediate release products: comparison of different bioequivalence experiments. *Basic Clin Pharmacol Toxicol.* 2005;96(3):265-7.
2. Klein S. The use of biorelevant dissolution media to forecast the in vivo performance of a drug. *AAPS J.* 2010;12(3):397-406.
3. Mircioiu C, Mircioiu I, Voicu V, Miron D. Dissolution-bioequivalence non-correlations. *Basic Clin Pharmacol Toxicol.* 2005;96(3):262-4.
4. United States Pharmacopoeia 30 – National Formulary 25, 2006.
5. Klein S, Dressman JB, Reppas C. In vitro methods to predict food effects. In: Amidon G, Lesko L, Midha K, Shah V, Hilfinger J. (eds.) International bioequivalence standards: A new era. TSRL Inc. 2006; Ann Harbor, USA.
6. Galia E, Nicolaides E, Horter D, Lobenberg R, Reppas C, Dressman JB., Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharm Res.* 1998;15:698-705.
7. Shah VP, Konecny JJ, Everett RL, McCullough B, Noorizadeh AC, Skelly JP. In vitro dissolution profile of water-insoluble drug dosage forms in the presence of surfactants, *Int J Pharm.* 1989;6(7):612-8.

Manuscript received: March 12th 2011