ORIGINAL ARTICLE

THE INFLUENCE OF COMPENDIAL CELLS DESIGN AND EXPERIMENTAL SETUP ON THE *IN-VITRO* SIMILARITY OF KETOCONAZOLE TOPICAL CREAMS

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Manuscript received: November 2014

Abstract

Two cream dosage forms containing 2% ketoconazole with significant similarity of their qualitative composition were comparatively analyzed by compendial tests using vertical diffusion cells and immersion adaption for the standard dissolution equipment. The mean *in-vitro* release profiles displayed marked differences between reference listed drug and the generic formulation, independent on the design of the cell and implemented operational parameters. These results corresponded to a marked non-similarity in terms of microstructure, as revealed by the hysteresis loop test. Considering that both topical semisolid formulations are approved by the regulatory authorities, probably based on clinical endpoint studies, the *in-vitro* data can be regarded as proofs of the overly discriminatory character of the methodology.

Rezumat

Două forme farmaceutice de tip cremă conținând 2% ketoconazol, prezentând o similaritate semnificativă a compoziției calitative, au fost analizate comparativ, pe baza unor teste compendiale, utilizând celule verticale de difuzie și adaptări pentru imersie, în cadrul echipamentelor standard de dizolvare. Profilele medii de cedare *in-vitro* au indicat diferențe majore între referința oficială și formularea generică, independent de tipul de celulă utilizată și de parametrii de operare implementați. Aceste rezultate au corespuns cu non-similaritatea accentuată în privința microstructurii, conform datelor reologice. Considerând că ambele produse au fost autorizate de către agenții de reglementare, probabil pe baza studiilor clinice, datele *in-vitro* pot fi interpretate ca dovezi ale caracterului supra-discriminatoriu al metodologiei.

Keywords: ketoconazole, semisolids, *in-vitro* release, vertical diffusion cell, enhancer cells, ointment cells

Introduction

The *in-vitro* release tests (IVRT) represent a powerful quality control for the evaluation of the combined influence of several composition and manufacturing variables. The methodology has been developed by analogy with dissolution tests, extensively applied for routine assessment of batch-to-batch consistency, as well as indicative, predictive evaluations for the *in-vivo* behavior. They were officially adopted in 1997 [1], when the United States Food and Drug Administration (US-FDA) issued to the *Guidance for Industry - Nonsterile Semisolid Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation* (SUPAC-SS). The initial applications were limited to the analysis of reduced changes in terms of qualitative or quantitative composition and modification of process parameters, as described under the level 2, i.e. changes that may impact the performance of topical semisolid dosage forms. The utility can be extended to selection of optimal formulation candidates, during the research and development phase, and to the assessment of lower or intermediate strengths, once the bioequivalence of the highest strength has been demonstrated by a regulatory acceptable approach.

In this context, it must be considered that the complexity of the topical semisolid dosage forms, but also the particularities of the biological barrier on which they are applied, makes it very difficult to develop an *in-vivo* methodology based on pharmaco-kinetic principles for bioequivalence assessment. Tape stripping or dermatopharmacokinetic technique has been proposed by US-FDA [2], but apparently divergent conclusions generated in two independent

clinical trials [3] leaded to the withdrawal of the corresponding draft guidance. Consequently, there is a continuous quest for a methodology able to reflect the rate and extent of penetration of active pharmaceutical ingredient after topical administration in semisolid vehicles. Except for corticosteroids for which the vaso-constrictor effect is used for indirect evaluation of local delivery [4], clinical end-point studies remain the main regulatory-recommended approach. Currently, there is a consensus that combined *in-vitro* and *in-vivo* methodologies could be the optimal selection, with accurate tailoring to the particularities of the disease, drug, product and site of action [5].

Two key moments can be identified in the continuously extending applicability of IVRT. The first one is the official adoption of a dedicated compendial General Information Chapter <1724> entitled Semisolid Products - Performance Tests by the United States Pharmacopeia [6]. Additional to the available SUPAC-SS provision, this chapter includes a detailed description of various types of diffusion cells reported in literature for the evaluations of release from topical lotions, gel, creams and ointments. There is an obvious tendency of the compendial forum to standardize the design of the experimental device, with a prospected adoption of single model, similar to paddle or basket apparatus. Except for three types of the vertical diffusion cells [7], all the other equipment represent adaptions to the standard dissolution systems, i.e. miniaturized stirring elements and vessels and fitted sample holders. The second key moment is the issue in March 2012 of the Acyclovir 5% ointment draft guidance by US-FDA [8]. The document mentions two options for demonstration of bioequivalence between reference listed drug (RLD) and a generic product: a clinical endpoint study, respectively *in-vitro* similarity. The latter option is feasible for similar qualitative and quantitative compositions and comparative physicochemical characterization. It is the simplicity of formulation (a dispersion of drug in polyethylene glycol, [9]) and the difficulties in demonstration of clinical effect that made the regulatory authority to accept IVRT as a more sensitive and *in-vivo* relevant methodology.

Although feasible, the *in-vitro/in-vivo* correlation is prospectively more difficult to achieve [3], due to the complexity of interactions between the topical semisolid dosage forms and the rate-limiting layer of the skin, the *stratum corneum*. The real value of IVRT, as a comparative quality assessment, is provided only after an adequate evaluation of composition differences able to change significantly the *in-vivo* exposure profile. The experimental setup also plays an important role in generating relevant data. The aim of the current paper was to analyze the impact of the difference in compendial diffusion cell design on the release profiles of ketoconazole from topical creams. Two semisolid products available on the market with minor compositional difference were compared using different *in-vitro* set-ups.

Materials and Methods

The qualitative composition of the semisolid dosage forms subjected to diffusional and structural evaluations is presented comparatively in Table I. The two creams containing 2% ketoconazole represented the official RLD (coded R) and a generic product (coded T), having the same inactive ingredients except for the preservative.

Table I

The qualitative composition of the evaluated products (information available on the package insert)

Product	R	T
Ketoconazole	✓	✓
Cetyl alcohol	✓	✓
Stearyl alcohol	✓	✓
Isopropyl myristate	✓	✓
Propylene glycol	✓	✓
Sorbitan monostearate	✓	✓
Polysorbate	✓	-
Polysorbate 60	-	✓
Polysorbate 80	-	✓
Butylated hydroxyanisole	-	✓
Sodium sulfite	✓	-
Purified water	✓	✓
Batch	9FB1P00	G0004

The IVRT protocol included a system of six vertical diffusion cells (VDC, Hanson Microette, Hanson Research Inc., US), with a declared volume of 12 mL (n = 6; net volume of approximately 10 mL, within cell variation of less than 5%). The assembly was heated to 32 ± 0.5°C, using a Lauda Star Edition thermostat (Germany). The receiver consisted of hydrochloric acid 0.1 N pH = 1.2, able

to provide sink conditions considering the basic characteristics and the low aqueous solubility of the drug. Additionally, hydro-alcoholic mixtures composed of 30 and 50% absolute ethanol in purified water (v/v) were screened. The media was degassed by filtration under vacuum through 0.45 µm cellulose acetate disk filters at room temperature (Sartorius Stedim Biotech, Sartorius GmbH, Germany). Artificial polysulfone membranes (Tuffryn®, PALL Life Sciences HT-450, 0.45 µm average pore size) were soaked into the receiver for half an hour, and blotted superficially with cellulose tissues. The studies were conducted in infinite dose, occluded conditions. Samples of 0.5 mL were collected manually at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours after initiation of the stirring (600 rpm, using magnetic bars with helix). The agitation was stopped 30 seconds before sampling.

For the immersion cells, two types of dissolution stations were used in combination with mini-paddles and flat bottom, low volume vessels. The Enhancer Cells (EC) in three designs, corresponding to variable volume and a surface area of 0.5, 2.0 and 4.0 cm^2, were mounted on an Agilent 708-DS Dissolution Apparatus (Agilent Technologies, US). The depth of donor compartment was adjusted in order to apply the same amount of creams, 500 mg, identically to the fixed quantity accommodated by the Ointment Cells (OC, Hanson Research Inc., US, using a SR8 Plus Dissolution Station). The cells were slowly immersed into 150 mL preheated media, using long tweezers, preventing the appearance of air bubbles. The mini-paddles were lowered to 10 mm above the surface of the membranes and the stirring was initiated at 50 rpm. The other operational parameters, including media composition, temperature and sampling times, were the same as previously described for VDC. The volume of samples was 2 mL and replacement with blank pre-heated media was applied. The sampling region was selected in the middle space between the upper part of the mini-paddle and surface of the receiver. The vessel covers were sealed with Parafilm® M laboratory film (Science Services, Germany), for prevention of evaporation losses.

The quantitative analysis of the released amounts of ketoconazole was determined spectrophotometrically (λ_{max} = 221 nm) after the appropriate dilution of samples, using an Agilent 8453 Pharmaceutical UV-visible system (Agilent Instruments, Germany) with Multicell transport tray and ChemStation G1116AA Advanced Software (Rev.B.04.01).

The individual release rates were calculated using the compendial approach (Higuchi model), as the slope of the regression lines performed on the dependence of amount release per surface area *versus* square root of time. 90% confidence intervals were calculated for test to reference slopes ratio, using the SUPAC-SS non-parametrical method and an acceptance interval for similarity of 75 to 133.33%.

The hysteresis loop test was performed for structural analysis on a Thermo Haake VT550 rotational viscometer (Thermo Electron GmbH, Germany) with SV - DIN spindle attachment (shear rate interval: 0 - 25 1/s; sample volume: 10 mL; n = 6), at room temperature.

The cream products, analytical standard of ketoconazole (purity higher than 98%, Sigma), hydrochloric acid 37% solution (Emsure®, Merck) and absolute ethanol (LiChrosolv®, Merck) were commercially purchased and used as received. The purified water was obtained using a SGW Ultraclear UV Plus™ equipment (Germany).

Results and Discussion

The experimental data confirmed the dependence of *in-vitro* release profiles on the testing conditions, particularly on the composition of the receiver and its capacity to solubilize the drug. The hydro-alcoholic mixtures were not adequate for providing sink conditions. A 30% or lower concentration of absolute ethanol in purified water generated highly variable, non-Higuchi release, probably due to rapid saturation of the receiver compartment and dependence of the release processes on the frequency of sampling. For 50% absolute ethanol, the variability was considerably reduced, but the amounts of ketoconazole were six times lower, compared to the acidic medium (Figure 1). Interestingly, the decrease of the release rates was similar for both products, the diffusion being limited by the concentration gradient across the membrane interface and not by the contact angle between the pre-conditioned membrane and the lipophilic vehicle.

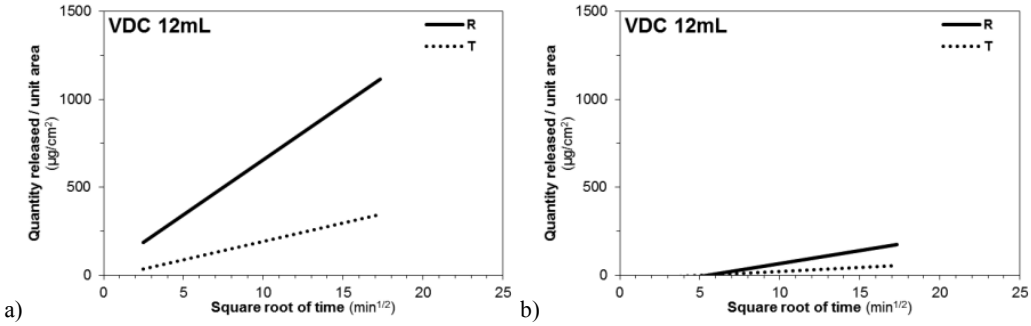

Figure 1.

The dependence of mean *in-vitro* release profiles of ketoconazole from 2% topical creams (n = 6) on the composition of receiver: a) hydrochloric acid 0.1N (pH = 1.2); b) ethanol absolute in purified water (1:1)

As mentioned previously, for revealing the impact of design variables on the *in-vitro* profiles, unlimited sink conditions are essential. Therefore, based on these preliminary results, hydrochloric acid 0.1 N pH = 1.2 was selected as receiver, considering the weak basic characteristics of ketoconazole [10]. Obviously, no biological relevance can be assumed for these conditions. The release rates were dependent on the thickness of the layer of formulations applied onto the artificial membrane. For example, in case of the reference product, the rates generated by the Enhancer Cells decreased from 142.79 µg/cm^2/min$^{1/2}$ for 0.5 cm^2 exposed surface to 80.48 and 64.43 µg/cm^2/min$^{1/2}$, for 2 and respectively 4 cm^2 (Figure 2). Comparable thickness and diffusional areas induced similar *in-vitro* profiles. The possible contributing factors include the distinct time intervals needed for temperature equilibration of the cream layer, as well as the variable degree of depletion in the donor compartment and the value of the dead volume [7, 11].

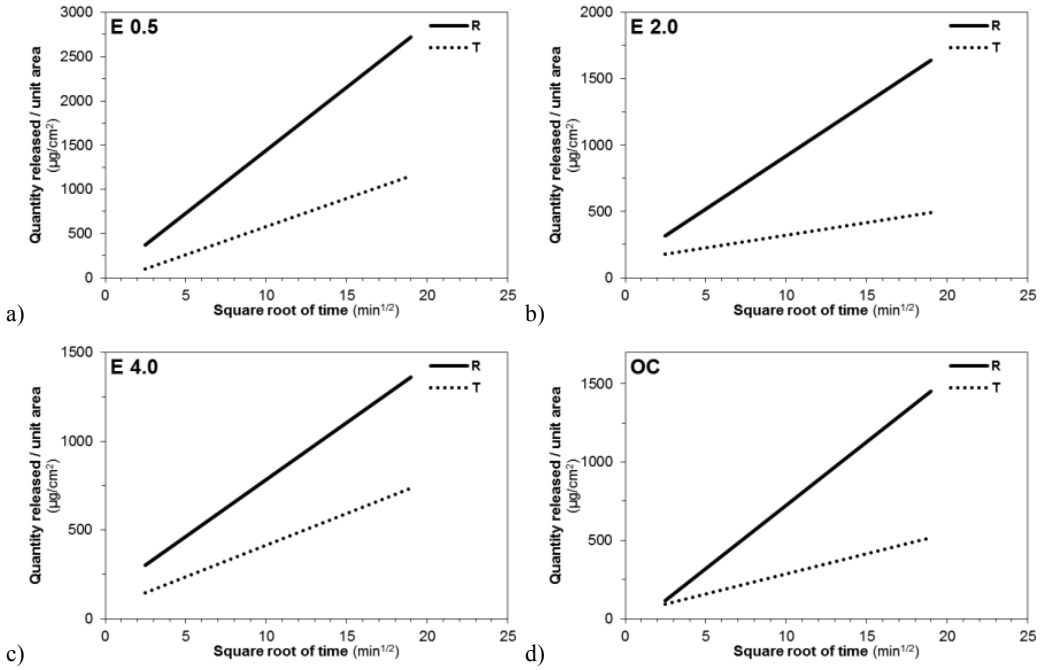

Figure 2.

The mean *in-vitro* release profiles of ketoconazole from 2% topical creams (n=6) obtained using various types of compendial immersion cells: a) Enhancer cells 0.5 cm^2 (E 0.5); b) Enhancer cells 2.0 cm^2 (E 2.0); a) Enhancer cells 4.0 cm^2 (E 4.0); a) Ointment cells (OC)

The difference observed between the mean *in-vitro* release profiles corresponded to the deformation pattern under the controlled shear stress. Both semisolid products presented a pseudoplastic behavior, with a lower consistency and ampler destructuration for RLD. A higher viscosity of the generic formulation (Figure 3) corresponded to an increased diffusional resistance and consequent slower release.

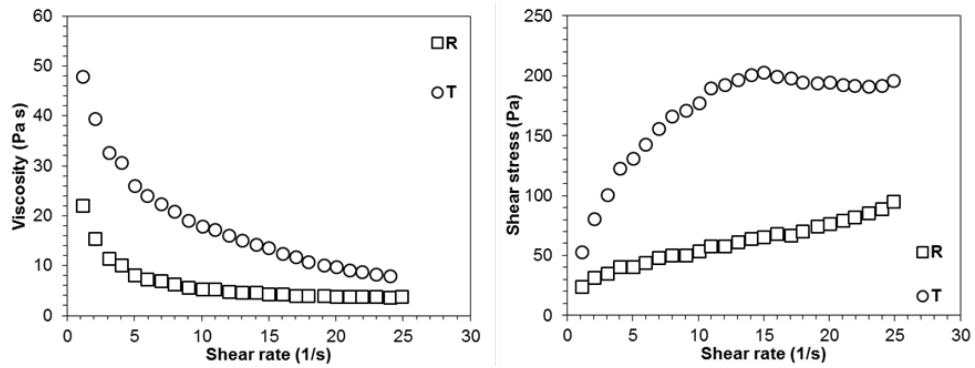

Figure 3.
The variation of viscosity and shear stress with the shear rate (mean profiles, n = 6; standard deviation was not displayed for the clarity of the graphs)

It is to be noted that the qualitative composition of the two formulations were essentially similar. The considerable structural differences are probably linked to the process variable and less likely to the amounts of various excipients. Segers J.D. *et al.* demonstrated that significant microstructural changes, revealed by IVRT, were induced by changes of manufacturing parameters, such as stirring speed in homogenization phase or cooling rate [12]. Moreover, the diffusional resistance may change for semisolid vehicles during storage and throughout the shelf-life of the product, without affecting the *in-vivo* performance [13]. An available technical note from US-FDA [14] suggests that a difference in spreadability as estimated by vane method (non-Q3 products) does not necessarily generate bioinequivalence.

Independently on cell design and operating parameters, *in-vitro* non-similarity was concluded based on the calculation of 90% confidence interval for the individual test to reference slopes ratio. The inter-cell variability of data was below 10%, once the steady state was achieved for the transfer across the membrane. The upper limit was below the lowest limit of the acceptance interval (Table II). This supports the discriminatory or overly-discriminatory character of the applied methodology, just as usually concluded for dissolution tests [15, 16]. The difference in the calculated values of the limits may attributed to the mass or volume ratio between donor and receiver compartment, as well as to the heating profile and partial depletion, depending on the thickness of the formulation layer.

Table II
The lower (LCL) and upper limit (UCL) of the 90% confidence interval calculated for the individual test (T) to reference (R) release rates, obtained in various experimental conditions (acceptance interval of *in-vitro* similarity: 75 - 133.33%)

Diffusion cell	Surface area (cm^2)	Quantity of formulation (mg)	Volume of the receiver (mL)	T/R[1]	LCL (%)	UCL (%)
VDC	1.767	300	10	33.69 (29.74)[2]	0.3040 (0.2573)[2]	0.3571 (0.3311)[2]
EC	0.5	500	150	46.04	0.4236	0.4652
	2.0			58.96	0.5506	0.6235
	4.0			55.53	0.5281	0.5810
OC	1.767			31.74	0.3019	0.3273

[1] slope ratio corresponding to mean *in-vitro* release profiles for the two creams containing 2% ketoconazole represented the official RLD (coded R) and a generic product (coded T); [2] calculated for the release rates obtained in hydro-alcoholic mixture.
VDC - vertical diffusion cells; EC - Enhancer Cells; OC - Ointment Cells; LCL – the lower limit of the 90% confidence interval; UCL - the upper limit of the 90% confidence interval.

The results confirmed that IVRT are able to reflect in aggregate the combined effect of several variables that affect the pharmaceutical quality of a topical semisolid dosage form, such as the micro-arrangement of the matter, particle size and shape or droplet size, solubility of the drug within the matrix etc. [1, 17]. However, it should not be considered as an accurate measure of bio-availability nor an indicator of bioequivalence. The antifungal drugs are supposedly delivered to a significant extent only in the outermost layer of the skin, the *stratum corneum*. This segment is frequently considered as the rate limiting compartment [18], but the penetration is strongly dependent on the pathological process intended to be treated, particularly on the degree of alteration. IVRT can be

regarded as a safety evaluation when the integrity of the *stratum corneum* is compromised. For ketoconazole, the lipophilic character and the relatively high concentration of the dosage form can be correlated with a saturation biological barrier which is at the same time the site of action. Using the *in-vivo* exposure profiles generated by an improved dermatopharmacokinetic protocol, N'Dri Stempfer B. *et al.* concluded that less than 10% of the applied dose of econazole 1% cream was recovered in adhesive tapes [19]. The registration of the evaluated products was probably based on results from clinical endpoint studies, which are known as less sensitive to both composition and manufacturing variables.

Conclusions

Two cream formulations containing 2% ketoconazole were comparatively tested using *in-vitro* release methodologies and rheological assessment. Despite the significant similarities in terms of qualitative composition, the products displayed different release rates, corresponding to distinct micro-arrangement of the matter as indicated by the hysteresis loop test. The variability in design between the compendial vertical diffusion cells and immersion cells didn't impact the conclusion on *in-vitro* non-similarity. Considering that adequate proofs of therapeutic equivalence were submitted as part of the abbreviated new drug application, the IVRT results can be considered as proofs of the overly discriminatory character of the implemented procedures.

Acknowledgements

The work has been funded by the Sectoral Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132395. The results have been partially presented at the International Symposium on Scientific and Regulatory Advances in Complex Drugs, Budapest, Hungary, October 27-28, 2014.

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