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THE IMPACT OF DIFFERENCES BETWEEN REGULATORY GUIDANCE FOR *IN VITRO* TESTING ON THE DISSOLUTION PROFILES OF MYCOPHENOLATE MOFETIL

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Abstract

The paper presents the results of *in vitro* dissolution testing for various immediate release solid oral dosage forms (three film coated tablets and one capsule formulations), containing 250 and 500 mg mycophenolate mofetil. The release profiles were evaluated according to the current recommendations available in the international biowaiver guidance, including three pH stages and two stirring rates. A very rapid and complete release was observed in the acidic conditions. The most discriminatory conditions were obtained when the acetate buffer pH=4.5 was used as release media. The low solubility of the drug substance pointed out the methodological limitation in non-sink conditions; mostly concerning the comparison of different dose strength.

Rezumat

Lucrarea prezintă rezultatele testelor de dizolvare *in vitro* aplicate diferitelor forme farmaceutice cu cedare imediată (trei formulări tip comprimate filmate, o formulare tip capsulă), conținând 250 și 500 mg micofenolat mofetil. Profilele de cedare au fost evaluate conform recomandărilor curente din ghidurile internaționale de exceptare de la studiile *in vivo*, pe trei nivele de pH și la două viteze de rotație. O cedare completă și rapidă a fost observată pentru studiile realizate în mediul acid. Cele mai discriminatorii condiții au fost înregistrate la folosirea tamponului acetat, pH=4,5 ca mediu de dizolvare. Profilul de solubilitate redusă a substanței active a evidențiat limitările metodologice ale condițiilor *non-sink*, mai ales la compararea diferitelor doze.

Keywords: mycophenolate mofetil, biowaiver, dissolution, sink conditions.

Introduction

The main drug regulatory authorities officially adopted the biopharmaceutical classification system (BCS) and the biowaiver principles

of *in vivo* bioavailability and bioequivalence, to a different extent [1]. Food and Drug Administration (FDA) issued the first and most conservative guidance, limiting the applicability to immediate release oral solid dosage forms, containing high permeability, high solubility drugs [2]. European Medicine Agency (EMA) [3] and World Health Organization (WHO) [4] extended the framework to class II and / or class III drugs, provided that particularly rapid release occurs (Table 1). Even since the underlying physico-chemical principles have been stated, the foreseen use of BCS as scientifically-based tool for adequate prediction of *in vivo* performance has not been fully accepted. Nevertheless, its use has been prospectively evaluated by the professional organization, especially by the International Pharmaceutical Federation, which periodically publishes new specific monographs [5]. The key issue is the implementation of the BCS-based biowaiver concept for granting a rapid and wide access of the population to high quality generic drug products. The time and funds consuming *in vivo* studies can be replaced by validated, surrogate *in vitro* methodologies, evaluating the critical steps of release after oral administration.

Table I

Comparative presentation of the official *in vitro* testing conditions and their regulatory applicability for biowaiver procedures

Authority	WHO [2]		FDA [3]		EMA [4]	
Apparatus	I ¹	II ²	I ¹	II ²	I ¹	II ²
Stirring rate (rpm)	100	75	100	50	100	50
Volume (mL)	900					
Class I (HP, HS)	✓		✓		✓	
Class II (HP, LS)	✓		✗		✗	
Class III (LP, HS) ³	✓		✗		✓	

¹basket apparatus; ²paddle apparatus; ³immediate release oral solid dosage forms must display a very rapid dissolution profile, i.e. >85% fraction dissolved within 15 minutes.

Abbreviations: HP - high permeability; LP - low permeability; HS - high solubility; LS - low solubility.

Beside the requirements of drug stability in the gastro-intestinal environment and lack of excipients potentially interfering with the absorption, one of the clear limitations refers to the critical group of narrow therapeutic index (NTI). Small variations in the exposure can trigger either the lack of the therapeutic effect or specific adverse drug reactions. Typical examples of NTI's include immunosuppressive drugs. Mycophenolate mofetil (MMF) is the prodrug of a selective inhibitor of inosine monophosphate dehydrogenase, mycophenolic acid [6]. The morpholino-

ethyl ester is typically used for the manufacturing of immediate release, oral solid dosage forms, whereas enteric-coated compositions include the sodium salt [7]. To this moment, the applicability of biowaiver principles for MMF has not been reported. It should be considered that, although an immunosuppressant, it is not clearly mentioned in the available lists of NTI's [8]. Moreover, the utility of therapeutic drug monitoring procedures, implying accurate quantification of concentrations in available biological fluids and consequent adjustments of dosage is still not clearly demonstrated [9].

The biopharmaceutical properties of MMF are dominated by its weak base character and the decrease in solubility at pH values corresponding to the distal regions of the gastro-intestinal tract. Of particular interest is the fact that, in contrast with the typical basic drugs, e.g. antifungal azoles [10], the solubility drop is less accentuated in the duodenal conditions (600 mg / L, in acetate buffer pH=4.5 [11]). Consequently, typical nucleation phenomenon may not occur, as a frequent case of *in vitro in vivo* non-correlations. Despite the lack of information on the biorelevance of regulatory recommended [12] or physiologically simulated dissolution conditions, several authors have speculated the possible therapeutic inferiority of *in vitro* non-similar formulations [11, 13]. None of those reports systematically considered the requirements of available biowaiver guidance.

The aim of the paper was to comparatively assess the dissolution profiles of various generic and innovator, immediate release solid oral dosage forms containing 250 and 500 mg MMF. The selected test conditions corresponded to the most permissive, respectively the most restrictive regulatory frameworks.

Materials and Methods

Three film coated tablets (C500 - the highest strength of the reference listed drug, M500 and O500, containing 500 mg MMF) and one capsule formulation (C250 - the lowest strength of the reference listed drug, containing 250 mg MMF) were included in the study.

The *in vitro* dissolution profiles were evaluated according to the recommendations provided in the current *biowaiver* guidance. The paddle apparatus (USP 2) was adapted and used at two stirring rates: 50 [3, 4] and, respectively, 75 rpm [2]. The capsules were introduced in standard stainless steel sinkers (23 x 12 mm), in order to prevent the floating phenomenon and to compare the release parameters in the same hydrodynamic context. The evaluations were performed on six units, using a Hanson SR8 Plus dissolution tester (Hanson Research Inc., US). 900 mL of three release

media (solution of hydrochloric acid, pH=1.2, acetate buffer pH=4.5 and phosphate buffer pH=6.8) were degassed and heated to 37°C. Manual sampling procedures were performed at 5, 10, 15, 20, 30, 45, and 60 minutes, the collected volume being filtrated through resident canulla filters (10 µm mean pore size).

The amount of mycophenolate mofetil in the collected samples was determined after dilution with the corresponding media (dilution factor 10). A spectrophotometric method (λ_{max} =305 nm) was validated for the interval of resulting MMF concentrations, using a 8453 Pharmaceutical UV-visible system, Agilent Technologies, with Multicell Transport systems and Advanced UV-Vis Software, Rev.B.04.01.

The comparison of mean dissolution profiles was based on the calculation of compendial metrics. The *in vitro* equivalence was concluded if the value of the difference factor was lower than 15, whereas the similarity factor was higher than 50. A single experimental point was considered above the 85% level of the fraction released.

All reagents were of analytical grade and were purchased from Sigma Aldrich. The mycophenolate mofetil standard and the pharmaceutical products (undisclosed trade names and batch numbers) were a gift from a local laboratory and had 99.8% potency, according to the specific monograph of the United States Pharmacopoeia.

Results and Discussion

An unusual pattern of *in vitro* dissolution was observed for the acidic stage, with almost complete release within the first 10 minutes. All the evaluated products complied with the previously reported two stage specification of the reference listed drug [11]. The fraction dissolved surpassed the 75% limit after 5 minutes and the 90% limit after 15 minutes. The recorded differences between the mean profiles were remarkably low, located mainly at first sampling point and highly dependent on the stirring rate (Figure 1). No significant variability was noted for the most discriminative conditions (50 rpm). A high volume of particles was generated by the rapid disintegration of the solid dosage forms, but a confounding coning effect was not experimentally proven.

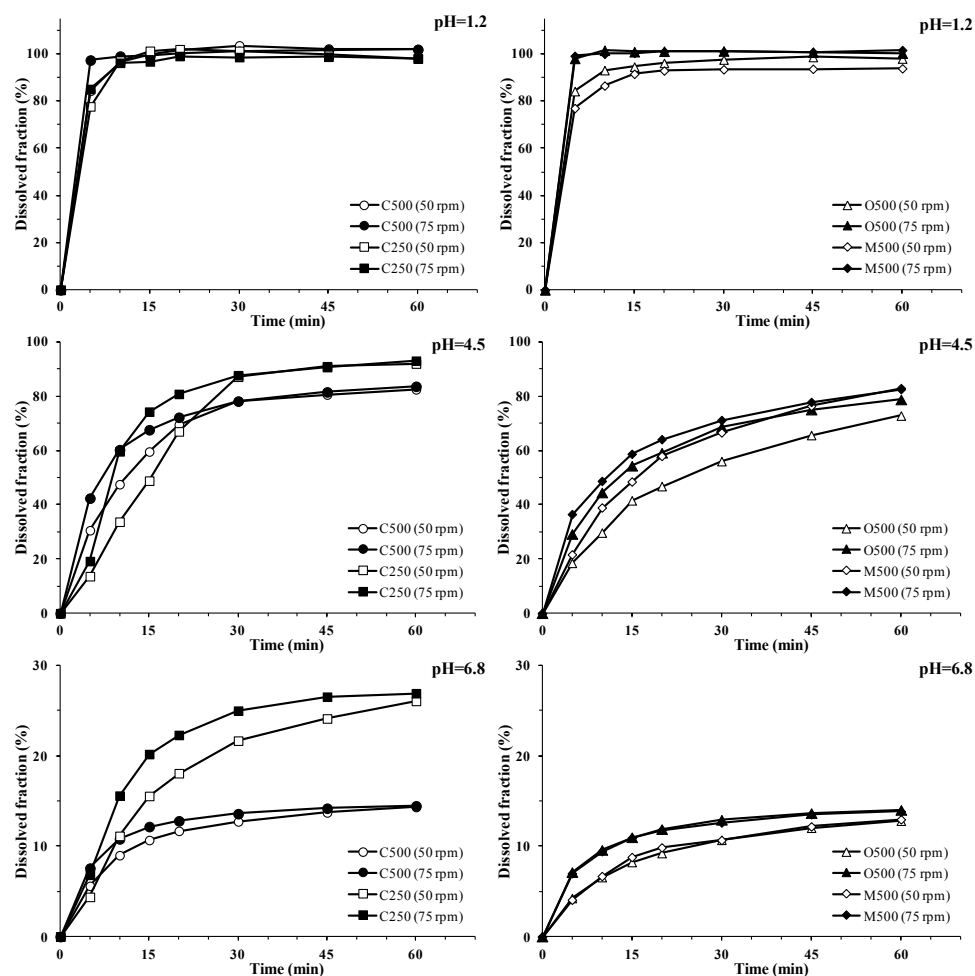


Figure 1.

The mean dissolution profiles of MMF from immediate release oral solid dosage forms in three media (n=6; standard deviation is not displayed, for clarity of the graphic)

It concerns the intermediate testing stage with pH value of 4.5, the solubility of MMF and the volume of media lead to an almost complete release, although the testing conditions didn't comply with the sink requirements. The profiles were critically altered by the hydrodynamic parameters and theoretically provide the most discriminatory conditions with respect to the possible non-similarities in qualitative and/or quantitative composition. The fraction released at the end of the tests varied between 73% and 93%, with no significant differences between the two levels of the stirring rate, for the same product.

The intestinal stage (pH=6.8) illustrated the importance of adequate screening of solubility profile for the active pharmaceutical ingredient in developing new drug release methodologies, especially when it is intended to be used for extrapolation of results between different doses. For the reference listed drug, even though there is no pharmaceutical equivalence between the two concentrations, the ratio of the amount released after 60 minutes is 1.86 for 75 rpm, approximately the same as the ratio of label-claimed doses. The solubility-limited profiles are less sensitive to the stirring rate throughout the test duration, except for the capsule formulation.

The comparisons of mean dissolution data were conducted only for the two buffer stages, since the acidic media generated less than three experimental points below the compendial level of 85% release (Table II). The profile corresponding to the highest dose of the innovator product was considered reference.

Table II

The comparison of *in vitro* release profiles based on compendial metrics

pH of media	Metrics	Stirring rate (rpm)	Test product			Reference product: C500
			C250	O500	M500	
4.5	f ₁	50	18.58*	26.13*	12.54	
		75	15.16*	16.69*	9.48	
	f ₂	50	48.53*	39.47*	53.75	
		75	47.52*	48.31*	58.01	
6.8	f ₁	50	58.73*	17.85*	16.09*	
		75	69.20*	6.31	7.50	
	f ₂	50	57.43	83.42	85.30	
		75	52.72	94.87	93.57	

* - values outside the acceptance interval for similarity

Unexpectedly, the 50% increase in the stirring rate according to the WHO recommendations [4] didn't change the conclusion of similarity, for the acetate buffer stage. It only leads to lower values of the difference factor, but still above the acceptance level of 15. For M500 product, with a single exception (f₁ value for 50 rpm in phosphate buffer pH 6.8), the *in vitro* release was equivalent to the innovator products, independent on the testing conditions or media composition. Noteworthy, the methodology has

some limitations in comparison of different dosage forms, mainly due to the distinct key processes that control the dissolution. The applicability of the compendial metrics requires the existence of the same mechanism of release.

Particularly for the phosphate buffer pH=6.8, the dissolution profiles are solubility-limited. The fraction released within one hour corresponded to the maximum value, considering the test conditions. It is probably one of the reasons for the specific recommendation introduced by EMA in the new bioequivalence guidance [3]. In cases where sink conditions may not be achievable for accurate comparison of two concentrations, the applicant may use equal doses. For MMF, this means that two capsules could be assessed *in vitro* and compared with higher strength. However, it is not clear how the hydrodynamics will be altered. Another observation available in the same guidance is that, without appropriate demonstration of possible *in vitro in vivo* correlations, the “*discussion .. [on] .. clinical / therapeutic relevance is considered inappropriate*”. The utility of *in vitro* dissolution tests as surrogate tools for bioequivalence and bioavailability assessment has already been proved [14]. The evaluated immediate release dosage forms are currently authorized in several countries and their bioequivalence with the reference listed drug has been proved, based on *in vivo* studies. Consequently, the implemented methodology could be considered overly discriminatory in these cases.

Conclusions

The impact of differences between the regulatory requirements included in the biowaiver guidance for *in vitro* testing on the dissolution profiles of mycophenolate mofetil has been evaluated, using several immediate release oral solid dosage forms containing mycophenolate mofetil. The experimental data indicated that the conclusion of similarity is not influenced by hydrodynamic parameters. The acidic stage didn't reveal any significant difference in release, due to the rapid disintegration of the formulations and to the high solubility of the drug. The acetate buffer media provided the most discriminatory conditions for the differences in the qualitative and quantitative compositions. Specific limitations in comparison of different concentrations in the non-sink media were reported.

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