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Exposing Profound Screening Potential of Ethanol-Based Dissolution Media in the Development of Oral-Modified Dosage Forms

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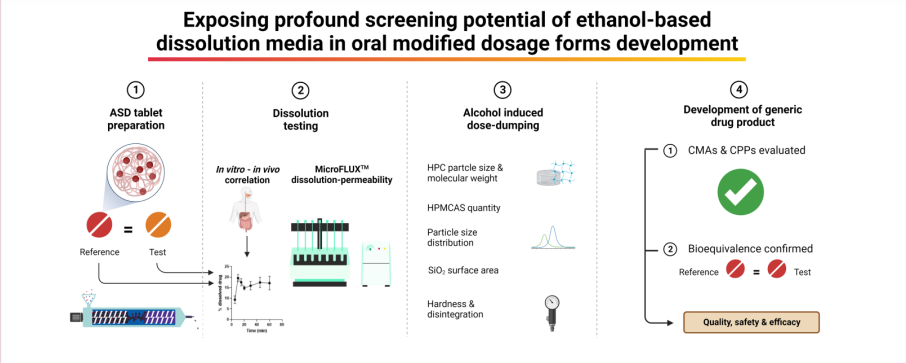
Abstract

Characterization of drug release from modified-release products in the presence of alcohol is required to evaluate the possibility of dose dumping due to safety assurance reasons. A generic product containing BCS IV drug and HPMCAS polymer was formulated as amorphous solid dispersion with hot-melt extrusion having the same qualitative and quantitative composition as the reference product. Although formulations were bioequivalent, they exerted different vulnerability towards ethanol, and the root cause was sought to establish appropriate control during product life cycle. To identify critical process parameters (CPPs) and critical material attributes (CMAs) according to the Quality by Design, a systematic screening of excipient properties and process parameters was performed. The in-depth evaluation revealed that dose dumping could be avoided with appropriate selection of specification limits for binder viscosity and particle size, specific surface area of the glidant and adjustments in the milling and tableting parameters. Due to the effective prevention of dose dumping, additional changes in qualitative or quantitative composition of the generic product were not required; thus subsequent bioequivalence study was not warranted.



Keywords: Dose-Dumping; Critical Material Attributes; Critical Process Parameters; Microfluxtm; Bioequivalence; Hot-Melt Extrusion

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Introduction

The control of drug release from modified release (MR) products mainly depends on the properties of polymer in the matrix or coating. Ingestion of MR product with alcohol can lead to alcohol induced dose dumping (AIDD), which refers to abrupt release of entire dose or a significant fraction thereof in a short period of time [1,2]. Regulatory considerations to prevent AIDD are reflected in the existing guidelines, which are not yet fully harmonized worldwide regarding the media that should be used (0.1 M HCl in US versus media used for routine testing in EU), the concentration of ethanol to be added (up to 40 % in US versus up

to 20 % in EU) or the duration of the dissolution test (up to 2 h in US versus not defined for EU) [1, 2]. Although it is desired to have rugged MR formulation with drug release being insensitive to the ethanol, this is not always possible [1]. Therefore, *in vivo* studies and/or risk assessments evaluating patient safety are justified. From generic perspective, to-be-marketed MR formulation should exert the same or superior resistance to ethanol (i.e. provide the same or slower release rate) compared to the originator's product [1].

In the assessment of formulation vulnerability towards ethanol one can expect ethanol causing changes in solubility, wettability, swelling, and mechanical properties of drug, excipients, and/or formulation [5]. Generally, ethanol increases solubility of hydrophobic drugs and in various ways affects performance of matrix forming excipients, which ultimately affect susceptibility of formulation to the media uptake, the onset of polymer swelling, and finally to deterioration of matrix/coating integrity and its resistance to erosion. On the other hand, the incoming material's porosity and compactness may also affect susceptibility of the surface to dissolution media and thus dose dumping [5]. All listed factors ultimately lead to altered drug release in the presence of alcohol [1,3-5].

While it could be argued that *in vitro* dissolution tests for assessment of AIDD may not capture *in vivo* bio-relevancy and are thus not predictive of *in vivo* situation owing to short-lived persistence of high ethanol concentrations in the stomach [3, 4], valuable information can be gathered with alcohol dissolution experiments regarding formulation performance, appropriateness of excipient selection [6], excipient critical material attributes (CMA), and critical process parameters (CPP). For generic industry such data may assist in the reverse-engineering process during development of bioequivalent products and in the Quality by Design strategy to establish appropriate control for the selected product.

The aim of this study is to present a case of MR generic product prepared with hot-melt extrusion that contains poorly soluble BCS IV model drug and hydroxypropyl methyl cellulose acetate succinate (HPMCAS) polymer as release-rate controlling excipient. The model drug exerts pH-dependent solubility (it is soluble in acidic environment), and very low saturated solubility in the physiologically relevant range. A pilot study was performed to establish *in vitro-in vivo* correlation between *in vitro* dissolution under non-sink conditions and *in vivo* results. With the established correlation, bioequivalent prototype on production scale was produced but dose dumping tests indicated that generic product would fail to pass AIDD regulatory requirements before entering the market. Therefore, an in-depth evaluation was triggered to identify potential CPPs and CMAs responsible for higher vulnerability of generic formulation towards alcohol and to establish control strategy in accordance with Quality by design to assure constant product quality/safety attributes through product life cycle without additional modifications in the qualitative or quantitative composition, since such alterations would necessitate additional bioequivalence study.

Materials and Methods

Materials

Excipients for extragranular phase were purchased from DuPont (microcrystalline cellulose - MCC), Ashland (hydroxypropylcellulose - HPC), DuPont (croscarmellose sodium -CCS), Grace GmbH (silicon dioxide) and Peter Greven (magensium stearate). HPMCAS was purchased from Ashland. Silicon dioxide was from Grace GMBH. Salts for dissolution media and solvents were of analytical grade (HCl, octanol, NaCl, NaH_2PO_4, NaOH) and were sourced from Merck KGaA, Germany, ethanol 96 % was sourced from Győr Distillery & Refinery Co., Hungary. SIF powder for FaSSIF media was from Biorelevant, United Kingdom.

Drug quantification was performed with UV on Microflux™ apparatus at 256 – 276 nm. HPLC quantification was performed for dissolution experiments; the method used was validated.

Methods

Microflux™

FaSSIF buffer solution pH 6.5 was prepared by dissolving 6.186 g of NaCl and 3.438 g of NaH_2PO_4 in 800 mL of distilled water, adjusting pH to 6.5 using 1 M NaOH solution. Next, 2.24 g of SIF powder and distilled water to 1000 mL volume was added and stirred for 2 hours.

Dissolution-permeability measurements were performed on Microflux™ apparatus, Pion Inc. Tablets were pealed, crushed and sieved. Fraction of 250 – 400 µm of crushed sample containing 2.5 mg of model drug was added into micro-dissolution vessels containing 10.0 mL of FaSSIF pH 6.5 at 37 ± 0.5 °C. Revolution was put to 500 rpm for the first 3 minutes to homogeneously disperse the extrudate, followed by adjustment to 100 rpm for the rest of the experiment. 10.0 mL of 1-octanol at 37 ± 0.5 °C was slowly added into the vessels and UV probes were immersed in 1-octanol for drug quantification with the *in-situ* Rainbow UV-VIS spectrophotometer, Pion Inc for the next 8 hours. Quantification was done using standardization curve (R^2 > 0.98) correlating drug concentration and AUC of 2nd derivative of the measured absorbance in the 256 – 276 nm wavelength interval.

Extrusion process

Model drug and HPMCAS were weighted in ratio 1 : 3 and blended with Cube Mixer, Erweka GmbH. Blend was sieved using Frewitt oscillating sieve 1.5 mm and feed in 18 mm twin screw extruder. Extrudate strands were pelletized, milled with hammer mill, blended with extragranular excipients and used for tableting.

Tableting

Milled extrudate and external excipients (silicon dioxide, HPMCAS, HPC, MCC, CCS, and magnesium stearate) in 2: 1 ratio were blended with Cube Mixer and compressed with single press tableting machine.

In Vitro Dissolution

Dissolution tests were performed on Agilent apparatus using paddle and 50 rpm. Samples were withdrawn at predetermined

time points every 15 min up to 2 h. Sampled aliquots were filtered through 0.45 µm PVDF filters discarding the first 5 mL of withdrawn sample. Withdrawn volume of dissolution media (10 mL) was not replaced.

Drug Release from Extrudate Samples

The amount of extrudate corresponding to one, two, or three doses were weighed, and 50 mL of testing media was added. 50 mL falcon tubes with extrudate and media were placed into shaker at 37 °C and 650 rpm. Samples were withdrawn, filtered through 0.2 µm MSVGN 2250 filter plates from Corning and the filtrate was diluted with dissolution media before HPLC analysis.

Saturated Solubility of Model Drug

Model drug (50 mg) was weighed into 15 mL tubes. After adding 10 mL of dissolution media, eprouvettes were placed into shaker at 37 °C and 650 rpm. Samples were withdrawn, filtered through 0.2 µm MSVGN 2250 filter plates from Corning and the filtrate was diluted with dissolution media before HPLC analysis.

In vivo bioequivalence study

A comparative bioavailability study was conducted under fast conditions to investigate the *in vivo* behavior and compare the bioavailability of the two test formulations to that of the reference product. The protocol for the study was approved by the relevant Independent Ethics Committee and was conducted in accordance with Good Clinical Practice and Declaration of Helsinki. Plasma concentrations of the active substance were measured by a validated, sensitive and specific LC/MS/MS assay. The pharmacokinetic parameters AUC (area under the plasma concentration time curve) and C_{max} (peak plasma concentration) were calculated using a non-compartmental approach.

All subjects were provided with an ICF in their language of preference for review. Prior to initiation of study procedures, the ICF was verbally reviewed with subjects by qualified staff, allowing sufficient time for review of the information provided and to answer any questions subjects had. Subsequently, subjects were informed of any developments or changes to procedures that could have influenced their continued participation in the study. Subjects did not anticipate any direct benefits from participation in this research study, with the exception that they received a health evaluation. Subjects who participated in this study were compensated for their time; however, they were not offered any incentives.

Results

Dissolution under Non-Sink Conditions to Establish *In Vitro – In Vivo* Correlation

Evaluation of drug release with compendial dissolution apparatus using 900 mL FaSSIF pH 6.5 as release media shows precipitation under non-sink conditions as shown in **Figure 1**. Since saturated solubility is attained within 15 min and high variability (RSD > 10 %) is evident throughout the test, dissolution in FaSSIF was found unreliable and not sufficiently rugged to guide prototype development. During drug release and due to extremely low solubility, phase transformation of dissolved drug is imminent and several different nano-species (i.e. nanodroplets, LLPS, crystalline seeds) were identified [7, 8], which cannot be completely separated from dissolved drug with currently available filter techniques. Data obtained with compendial dissolution approach is therefore highly variable. Also, with the concomitant occurrence of several nano-species and dynamic equilibria among them, questions regarding which specie is absorbed and which constitutes drug depo that drives the absorption remain unanswered. Thus, instead of monitoring amount dissolved in the aqueous phase, the amount distributed into organic phase was evaluated by using Microflux™ (**Figure 2**), and an excellent correlation between *in vitro* accumulation of dissolved drug in 1-octanol and *in vivo* pilot study results **(Table 1)** was established. The pilot study was performed using two test products (Test product 1 and Test product 2), which differed in the extruder configuration but have the same qualitative and quantitative composition as reference product. Based on favorable *in vivo* results seen with Test product 2, selected extruder configuration was used to prepare Test product 3 on scale-up on as to-be-marketed product. However, due to processability issues during scale-up extrusion, silicon dioxide was added into the extruding mass to avoid process stoppages. Nevertheless, biorelevant dissolution method in the Microflux™ indicated that this change would not impact formulation bio-performance.

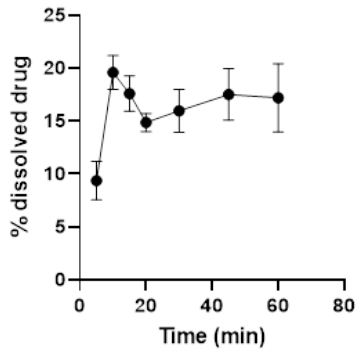


Figure 1 Typical dissolution profile of one tablet (n = 3) in 900 mL of FaSSIF pH 6.5 media, Apparatus 2, 100 rpm.

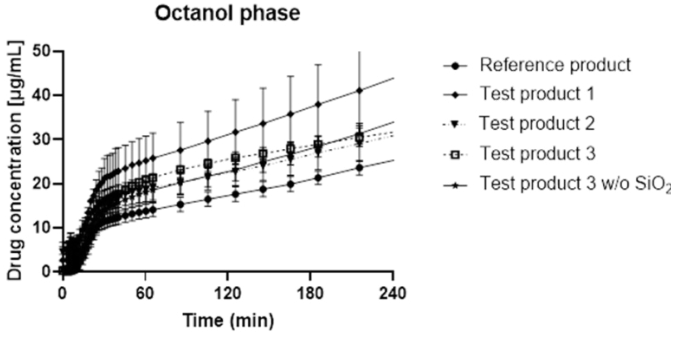


Figure 2 Permeation of dissolved drug from aqueous to organic phase – figure shows drug concentration in the organic phase.

Selection of the Most Discriminatory Dose-Dumping Dissolution Conditions

Test product 2 was bioequivalent **(Table 1)**. It also conformed to requirements regarding dose dumping; the release from generic Test product 2 was similar or slower/lower than the one of reference product used in the *in vivo* pilot study. Interestingly, the release was the highest in media with 20 % ethanol, not as one would expect in the media with the highest ethanol concentration of 40 % **(Figure 3)**.

However, when Test product 3 was screened against reference procured for the pivotal bioequivalence study, dose-dumping for the generic formulation in media with 20 % of ethanol was recorded **(Figure 4)**. Conditions with 10 % ethanol were thus not evaluated.

Based on evaluations in media for AIDD, 20 % of ethanol was selected as the most discriminatory dissolution condition that is sensitive to certain formulation and/or process parameters, which trigger dose dumping. Also, we observed that the reference product itself does not perform in a uniform and rugged manner, since reference lot used in pilot study and the one intended for pivotal study did not provide similar release in 20 % ethanol media.

Drug Substance Solubility in Proposed Ethanolic Media

Saturated solubility of model BCS IV drug in media used during dose dumping was evaluated **(Table 2)** and a theoretical number of doses that could completely dissolve were calculated. Based on presented results, drug solubility increases with the increasing amount of ethanol and in all cases, solubility is sufficient to allow for complete release. Furthermore, sink conditions are assured across all media (i.e. at least 3 doses would dissolve in 900 mL), thus, complete release 900 mL of each media is possible.

Table 1. *In vivo* results (average value and 95% CI) for pilot study under fast conditions for generic product.

Test product	C_{max}	$AUC_{t-\infty}$
1	126 % (117 – 136 %)	118 % (112 – 124 %)
2	115 % (106 – 125 %)	111 % (105 – 118 %)

Solubility of Extrudate With and Without Silicon Dioxide in Proposed Ethanolic Media

Since the only qualitative difference between Test product 2 and Test product 3 lies in the silicon dioxide addition into the extrudate to improve HME processability, drug release from extrudate sieved fraction 100 - 125 µm, was monitored. The amount of drug dissolved was tested at different initial extrudate amounts, corresponding to 1, 2 and 3 tablets being dissolved in 900 mL to evaluate if drug release is limited by the polymer solubility. Because drug solubility in 0.1 M HCl and ethanol is not limiting, any amount dissolved from the extrudate would be direct reflection of extrudate surface properties, wettability and/or resistance of HPMCAS matrix to dissolution media **(Figure 5)**.

Several surprising effects were noticed. Firstly, no difference in dissolution was recorded between both compositions, confirming that inclusion of silicon dioxide during extrusion is not causing dose dumping of the finished formulation **(Figure 4)**. When dissolution media with 10 % - 40 % ethanol was used, complete release was recorded within 24 h, which is also in accordance with high drug saturated solubility results.

Based on presented results, HPMCAS in the extrudate does not prevent AIDD and a complete release would be expected. Namely, in 2-h time-frame, as prescribed for dose dumping tests, approximately 10 % (in 0.1 M HCl), 50 % (in 10% ethanol in 0.1 M HCl), and complete release (in 20% and 40% ethanol in 0.1 M HCl) would be anticipated according to results in **Table 3**. The

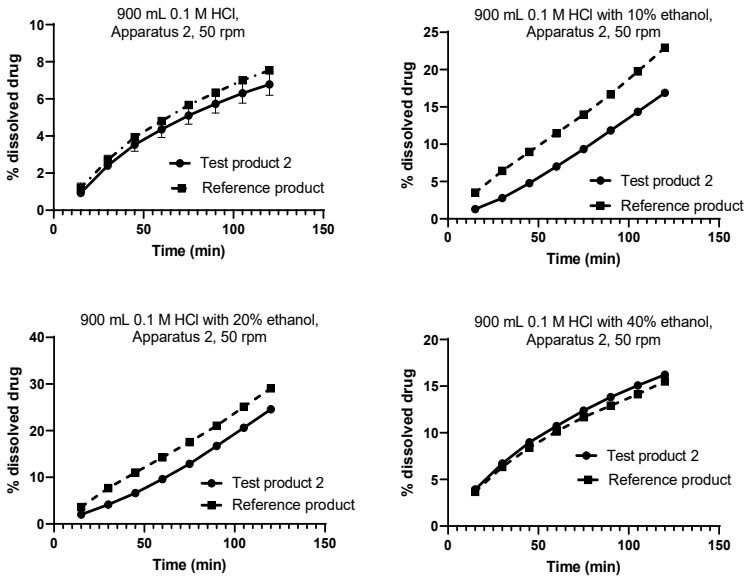


Figure 3 Drug release form Test product 2 and Reference product in media required to demonstrate the absence of dose-dumping. Graphs show average values of n = 3 repetitions for every sample with corresponding standard deviations.

Table 2. Solubility of drug substance in dose dumping media. Table shows average solubility and the number of doses that could completely dissolve in 900 mL.

Time (h)	0.1 M HCl		0.1 M HCl + 10% Et		0.1 M HCl + 20% Et		0.1 M HCl + 40% Et	
	Conc (mg/mL)	number of doses	Conc (mg/mL)	number of doses	Conc (mg/mL)	number of doses	Conc (mg/mL)	number of doses
0.5	0.6	5	2.2	19	3.3	29	5.5	50
1	1.0	9	2.2	19	4.6	42	5.4	50
2	1.0	9	2.2	20	4.7	42	5.4	50
4	1.0	9	2.2	20	4.7	42	5.4	50
6	1.0	9	2.2	20	4.8	43	5.3	50

Et – ethanol

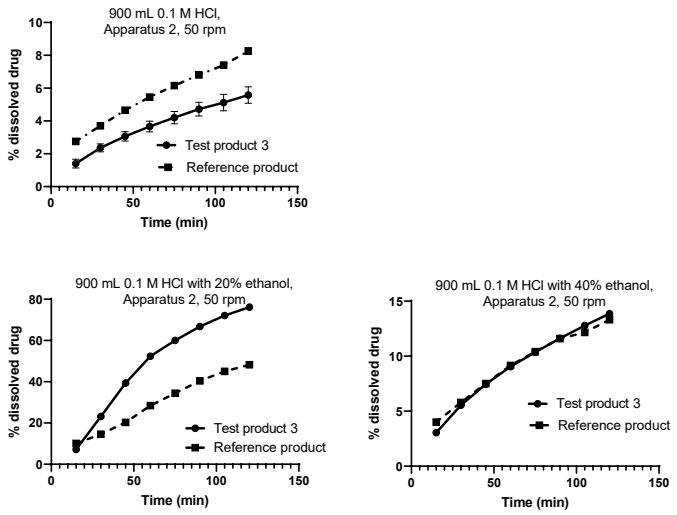


Figure 4 Drug release form Test product 3 and Reference product in media required to demonstrate the absence of dose-dumping. Graphs show average values of n = 3 repetitions for every sample with corresponding standard deviations.

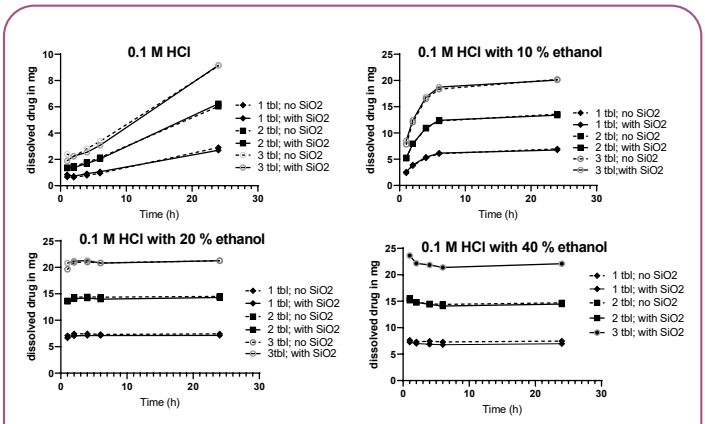


Figure 5 Impact of starting amount of extrudate on the % of drug released in proposed ethanolic media for extrudate with and without intragranular silicon dioxide, fraction analyzed 100 – 125 µm.

Table 3. The % of drug dissolved in each proposed ethanolic media for extrudate with and without silicon dioxide. Data corresponding to 1 tablet being dissolved in 900 mL of individual media are shown. The amount remaining at the end of experiment was quantified and the % based on the initial mass is reported.

	without SiO_2				with SiO_2			
Time (h)	0% Et	10% Et	20% Et	40% Et	0% Et	10% Et	20% Et	40% Et
1	11	34	95	102	13	38	93	104
2	**10**	**54**	**100**	**98**	**12**	**58**	**99**	**101**
4	12	75	100	100	15	80	100	99
6	15	87	99	98	17	91	100	97

discrepancy in drug release in the corresponding media between pure extrudate and finished dosage form (regardless of silicon dioxide) can thus only stem from down-stream processing after hot-melt extrusion process.

Mechanical Properties of Extrudate and Compactness

Tablets were compressed from both extrudates (i.e., with and without silicon dioxide) and dissolution in 20 % ethanol media was performed. Dissolution was compared between both compositions for tablets with similar tablet hardness and/or similar disintegration **(Figure 6 and Table 4)**. Dissolution results show that samples are ranked according to disintegration time within each composition – the shorter disintegration, the faster drug release. However, tablets that contain silicon dioxide show significantly shorter disintegration time than samples without it in spite of having similar tablet hardness (i.e. sample A is faster than D, sample B is faster than E). Overall, dissolution data and physical properties of analyzed tablets indicate that mechanical properties between both compositions are different.

Impact of Extrudate Particle Size

After extrusion extrudate is milled twice, first through 2.0 mm mesh, followed by the second step of milling through 0.5 mm mesh. The impact of different milling speeds on D_{50} values of extrudate were evaluated for both compositions **(Figure 7)**. Also, dissolution in 20 % ethanol was evaluated to test, if D_{50} also affects drug release from tablet cores.

The milling study indicates that under the same milling speeds significantly bigger extrudate particles are obtained when silicon

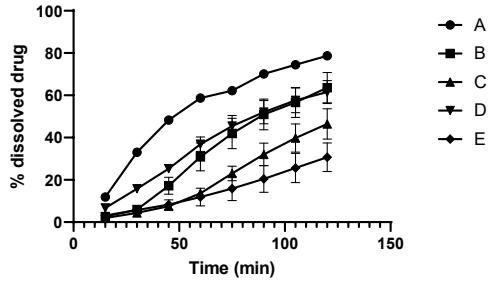


Figure 6 Impact of tablet hardness and disintegration time on drug release in 20 % ethanol in 0.1 M HCl (Apparatus 2, 50 rpm, 900 mL of 20 % ethanol in 0.1 M HCl) for tablets prepared from extrudate with and without silicon dioxide. Dissolution profiles were generated on n = 3 tablets for each sample. Data are shown as average values with corresponding standard deviations.

Table 4. Average tablet hardness and range of disintegration time for tablets prepared from extrudate with and without silicon dioxide.

Sample	Tablet hardness (in kp)	Disintegration time (in min)
A – with SiO_2	8.9	7-8
B – with SiO_2	13.9	13-17
C – with SiO_2	12.8	18-19
D – without SiO_2	11.0	12-14
E – without SiO_2	13.5	19-20

dioxide is present in the extrudate (see Appendix), indicating different mechanical properties. Furthermore, bigger extrudate particles also give faster and more variable dissolution in 20 % ethanol.

Impact of Hydroxy Propyl Cellulose Particle Size and Molecular Weight

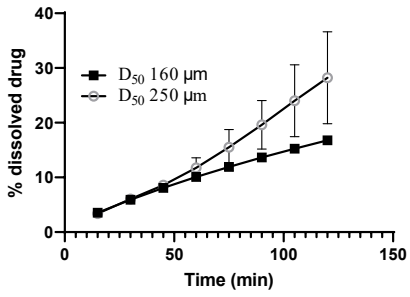
Hydroxypropyl cellulose (HPC) is one of the excipients used in the extragranular phase necessary for tablet compressibility since it acts as binder. Dissolution data in 20 % ethanol in 0.1 M HCl confirms that particle size of this excipient is crucial for efficient prevention of AIDD and smaller HPC particles provide better resistance to ethanol for two evaluated grades **(Figure 8)**. Besides particle size, molecular weight (at the same particle size of excipient) was also found to impact dose dumping **(Figure 9)**.

Impact of HPMCAS Quantity

HPMCAS is included in the extragranular phase to enable tableting by acting as binder. Omission of HPMCAS from extragranular phase leads to significant increase in drug release in 20 % ethanol in 0.1 M HCl, therefore this excipient is crucial to retaining tablet consistency and resistance to dose dumping **(Figure 10)**.

Impact of Silicon Dioxide Specific Surface Area

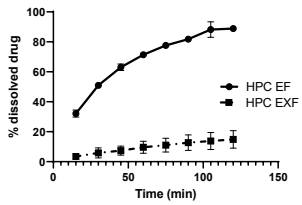
Extragranular addition of silicon dioxide as glidant enables continuous tableting process. Release in the presence of ethanol

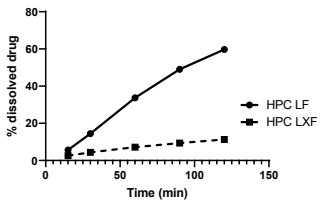


	D_{50}	
Milling speed	with SiO_2	without SiO_2
1100//3500	210	180
2000//3500	260	210
2000//5000	170	160
4000//3500	210	180

D_{50}	Tablet hardness (kP)	Disintegration time
160	14	14 - 16 min
250	12	20 - 24 min

Figure 7 Left - Impact of extrudate particle size on drug release in 20 % ethanol in 0.1M HCl (Apparatus 2, 50 rpm). Tablet cores were analyzed in n = 3 repetitions, graph shows average values with corresponding standard deviations. Right – impact of milling speed on extrudate particle size depending on the presence of silicon dioxide in the extrudate (SiO_2).c





HPC	PSD specification
LF, EF	total through 595 µm: 85 to 100%
LXF, EXF	total through 250 µm: 99.9%

Figure 8 Impact of HPC particle size on drug release in 20 % ethanol in 0.1 M HCl media (Apparatus 2, 50 rpm, 900 mL of 20 % ethanol in 0.1 M HCl). Graphs show average values of n = 3 repetitions for every sample with corresponding standard deviations. On the right side is table with PSD limits for used grades.

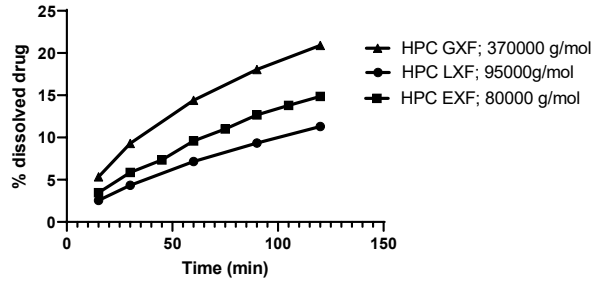


Figure 9 Impact of HPC molecular weight on drug release in 20 % ethanol in 0.1 M HCl media (Apparatus 2, 50 rpm, 900 mL of 20 % ethanol in 0.1 M HCl). Graph shows average values of n = 3 repetitions for every sample with corresponding standard deviations.

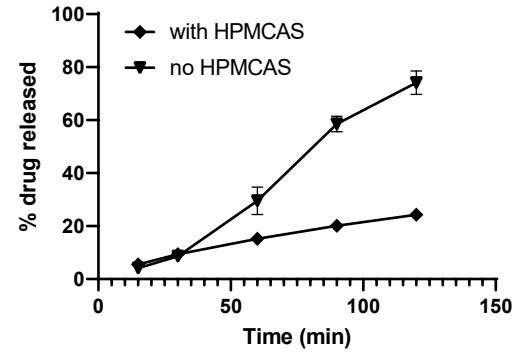


Figure 10 Impact of HPMCAS quantity in extragranular phase on drug release in 20 % ethanol in 0.1 M HCl media (Apparatus 2, 50 rpm, 900 mL of 20 % ethanol in 0.1 M HCl). Graph shows average values of n = 3 repetitions for every sample with corresponding standard deviations.

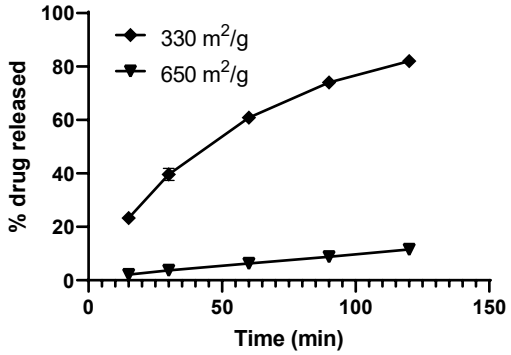


Figure 11 Impact of specific surface area of silicon dioxide in extragranular phase on drug release in 20 % ethanol in 0.1 M HCl media (Apparatus 2, 50 rpm, 900 mL of 20 % ethanol in 0.1 M HCl). Graphs show average values of n = 3 repetitions for every sample with corresponding standard deviations.

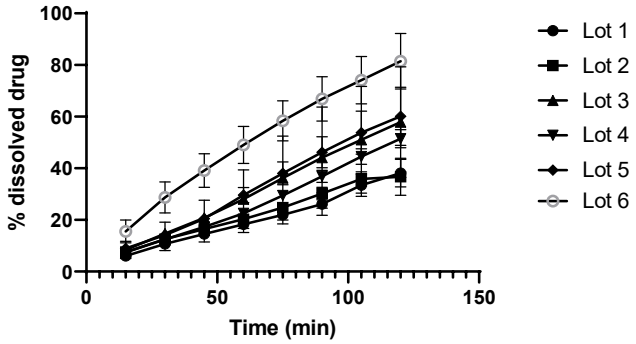


Figure 12 Drug release in 20 % ethanol in 0.1 M HCl media (Apparatus 2, 50 rpm, 900 mL of 20 % ethanol in 0.1 M HCl) from different reference lot numbers. Graphs show average values of n = 3 repetitions for every sample with corresponding standard deviations.

was evaluated for tablets where the impact of specific surface area of silicon dioxide in the extragranular phase was varied. Based on **Figure 11**, the impact is profound and higher specific surface area is desired to decrease formulation vulnerability towards alcohol.

Drug Release from Reference Product

Several different lots of reference product were screened for drug release in 20 % ethanol in 0.1 M HCl media **(Figure 12)** and significant differences among lots were identified (see Appendix). Also, some references exerted significant variability (RSD > 10 % at every time-point in dissolution profile), while others gave consistent results (Lot 2 and Lot 4). Since the composition of reference product is constant, the most probable cause for such differences could be poorly controlled process parameters or overlooked critical material attributes in the extragranular phase.

Discussion

Solubility of model BCS IV drug is pH dependent and in the physiologically relevant range limited solubility prevents the entire dose to dissolve in the compendial 900 mL of dissolution media. In ethanol however, drug solubility is sufficient to allow complete release. To improve therapeutic efficacy of the model drug, HPMCAS polymer is used as the carrier to obtain amorphous solid dispersion by hot-melt extrusion process. This polymer is also essential for prevention of drug crystallization on storage and stability, and it acts as inhibitor of precipitation during drug dissolution *in vivo* [9]. Also, HPMCAS polymer itself exerts pH dependent solubility and enables drug release in the environment with pH above 5.5 – 6.8, depending on the degree of substitution of succinoyl and acetyl groups [10, 11].

Due to the opposing solubility characteristics of weakly basic model BCS IV drug and slightly acidic HPMCAS, and very low drug solubility in physiologically pH range, guiding product development with compendial dissolution in bio-relevant media such as FaSSIF pH 6.5 has limited potential due to the interplay of dissolution, supersaturation, and precipitation **(Figure 1)**, which contribute to poor ruggedness of dissolution method. Therefore, dissolution tests to guide development of bioequivalent product

and establish *in vitro – in vivo* correlation were executed with Microflux™ apparatus. This experimental set-up enables *in situ* monitoring of dissolution on one side in aqueous media with or without surfactants (i.e. such as SIF powder). Dissolved drug then permeates lipophilic membrane into the receiver compartment that contains acceptor media with high concentration of sodium lauryl sulphate, which assures sink conditions and prevents precipitation. However, for the purpose of this product, slight deviation from the envisioned use was selected. Namely, the use of lipophilic membrane was omitted and a combination of FaSSIF pH 6.5 and 1-octanol as receiver media above FaSSIF was used. Without lipophilic membrane an additional rate-limiting step (i.e. membrane permeation) was avoided and better discriminatory power was obtained. The amount of accumulated dissolved drug in 1-octanol was monitored to reflect changes of drug in plasma, while quantification in FaSSIF phase was not performed due to extensive precipitation into various species (i.e. crystalline substance, nanoparticles, LLPS) [7, 8]. With this method *in vitro - in vivo* correlation was established according to the outcome of pilot bioequivalence study on healthy human volunteers under fast conditions. Fast conditions were correlated because they were confirmed to be more discriminatory. Two samples of generic product with the same qualitative and quantitative composition as reference product but produced with two different extruder configurations were tested *in vivo* **(Table 1)** and Test product 2 was found bioequivalent. Based on *in vivo* results and *in vitro* profiles of dissolved drug in 1-octanol a correlation was established. Namely, dissolution method developed with Microflux™ correctly ranked Test product 1 and Test product 2 compared to reference product according to corresponding C_{max} values. Thus, Test product 3 – final prototype sample with selected extruder configuration was manufactured on scale-up as the final formulation. However, due to processability challenges on production scale HME equipment, silicon dioxide had to be added into the extrudate of Test product 3 to prevent stoppages of the process. Regardless of this change, dissolution under non-sink conditions in Microflux™ confirmed that Test product 3 would be bioequivalent **(Figure 2)**.

However, when submitted to AIDD *in vitro* dissolution testing Test product 3 failed to provide similar dissolution as reference, although Test product 2, which was manufactured on the same extruder configuration but without silicon dioxide (Test product 2) passed **(Figures 3 and 4)**. Initially it was suspected that addition of silicon dioxide to extrudate of Test product 3 could be accountable for dose-dumping, since this change is the only difference among Test products 2 and 3. Namely, when silicon dioxide is added to extrudate, it can affect drug release as reported by Xia et al. [12]. Their study evaluated the impact of silicon dioxide positioning and noticed significant changes in itraconazole release in aqueous media when silicon dioxide was present in the extrudate, because it affected the performance of the polymer in solid dispersion by inducing polymer gelling and formation of a transition zone with higher viscosity. Also, it interacted with the drug in the solid dispersion, and it influenced wetting properties, which were observed as poorer deagglomeration of solid dispersion particles during dissolution. Slower penetration of dissolution media into the formulation, and diminished interactions between dissolution media and the drug were also identified as contributing factors to slower release. While their study noticed retardation of drug release, dissolution in FaSSIF pH 6.5 in our case remained unaffected. Differences were observed when dissolution in 20 % of ethanol was done, but they were not caused by inclusion of silicon dioxide, because release of model drug from both extrudates (with and without silicon dioxide) in ethanol was the same **(Figure 5 and Table 3)**. Therefore, the root cause for dose-dumping had to be identified in extrudate properties, which alone or in combination with extragranular excipients and/or down-stream processing steps affect drug release.

Process steps after hot-melt extrusion include milling, blending with extragranular excipients, tableting and film-coating. Having extrudate with different mechanical properties could influence every of the above-mentioned processes. In order to retain qualitative and quantitative composition of bioequivalent Test product 3 and simultaneously prevent AIDD, Quality by Design principles were followed to identify possible process steps (i.e. CPPs) and extragranular excipients properties (i.e. CMAs) responsible for AIDD and establish appropriate product control to avoid jeopardizing patient safety.

Results confirmed that milling and tableting outcome depend on the presence of silicon dioxide. The milling study showed that under identical milling conditions (i.e. mesh size and milling speed), extrudate prepared with silicon dioxide yields bigger particles of amorphous dispersion, which also enable faster and more variable release in ethanol media **(Figure 7)**. Furthermore, during tableting, tablet cores compressed from extrudate manufactured with silicon dioxide exerted shorter disintegration time than cores compressed with the same hardness from the extrudate that did not contain silicon dioxide **(Figure 6)** although in both cases identical extragranular mixture of excipients was added. Overall, both CPPs indicate that mechanical properties of the extrudate of Test product 3 were influenced by inclusion of silicon dioxide. When extruded, HPMCAS is known to have poor compressibility, because hot-melt extrusion with its heating and shear stress affects polymer orientation in a way that yields high extrudate particle density with increased dynamic hardness, making HPMCAS extrudate material less amenable for compression [13]. Therefore, any manipulations done to the extruded material that affects mechanical properties would be expected to affect processibility of the material such as milling and tableting. Also, bigger HPMCAS extrudate particles have less contact areas available for particle-particle interactions during tableting (i.e. bonding area), which favors inflow of dissolution media and thus drug release [14]. This presumption was also corroborated with AIDD results obtained for the tableting process.

On the other hand, AIDD outcome can also be controlled by adjusting the properties and/or quantities of excipients selected for the extragranular phase (CMAs). We have shown that AIDD release depends on the properties of binder in the extragranular phase. Namely, molecular weight and particle size of hydroxypropyl cellulose (HPC), when carefully selected, prevent AIDD **(Figures 8 and 9).** This is in accordance with Johnson et al [15] study that evaluated the impact of ionic strength on HPC swelling, gelation,

and disintegration time of compacts prepared from HPC and model drug. They confirmed that smaller particle size and lower molecular weight HPC resist premature disaggregation and the loss of HPC matrix integrity more efficiently, therefore enabling slower drug release. This is also in line with presented results obtained in 20 % ethanol evaluation in this paper, where ionic strength changes with addition of ethanol, which could explain our observations. Additionally, Johnson et al. [15] found that the relationship between drug release and ionic strength follows biphasic patterns (i.e. slower release at low and high ionic strength but faster release in medium ionic strength), which also explains why the fastest release in our study was observed with medium ethanol concentration of 20 % (and not at 40 %). Besides HPC, the investigated formulation in our case also contains HPMCAS in the powder form in the extragranular phase to assist in improving binding capacity between extrudate particles during tableting. The importance of HPMCAS role in the extragranular phase was demonstrated by significant dose-dumping when it was omitted from the formulation, therefore, the quantities of this excipient were not changed (Test product 2 and 3 retain the same quantity and quality – grade). Decreasing vulnerability of formulation towards alcohol with this excipient is logical, because HPMCAS is insoluble in acidic media and in this manner assists in retaining tablet integrity during dissolution as observed in patent application by Kanamaru et al. [16]. Their study corroborates that the presence of HPMCAS as well as particle size contribute significantly to mechanical resistance of sustained-release tablets under 200 rpm at pH 2.0. Silicon dioxide in the extragranular phase performs as glidant and it was also investigated as potential excipient whose specific surface area could impact AIDD release. We confirmed that the grade with bigger specific surface is better in preventing of AIDD.

Overall, several key points were identified that with carefully establish control could be applied in the production of generic product and whose overall effect could contribute to lowering sensitivity of generic formulation towards AIDD. Interestingly, when different reference lot products sourced from different markets were subjected to AIDD testing in 20 % ethanol **(Figure 12)** the same issue was identified. Some of tested originator lots showed dose-dumping effect indicating the absence of suitable control of CPPs/CMAs. Since the regulatory requirement states that dose-dumping of generic product must be compared to the dissolution profile of reference batch used in the bioequivalence study, superior quality pertaining to safety aspect is assured for the generic product presented in this paper.

Conclusion

This study presents the commonly overlooked potential of AIDD dissolution tests as one of crucial methods capable of explaining the complex interaction of CQAs, CMAs and CPPs in development of safe and effective final pharmaceutical dosage form. Also, it reveals gaps between the regulatory requirements and actual market situation. Namely, AIDD testing is a one-time *in vitro* study, performed on one reference batch. However, a systematic screening of different reference lots revealed poor resistance of some reference batches to the ethanol assuming the lack of systematic CPPs/CMAs control by the originator. Since the reference batch procured for the bioequivalence study exhibited low drug release in 20 % ethanol, generic product was systematically designed to assure low drug release in a consistent manner regardless of the lot-to-lot variability.

Appendix

Impact of SiO_2 on particle size distribution (PSD)

In order to assess, if the presence of SiO_2 in the formulation significantly influences the PSD of the granulate after milling; we have analyzed the data from **Figure 7**. Namely, the D_{50} represents the median of the PSD, therefore the significant difference in the D_{50} values with and without SiO_2 show that there is a significant shift between the distributions with respect to the presence of the SiO_2. There is no evidence that the difference of D_{50} values obtained from the granulates which differed only in the presence of the SiO_2 is not normally distributed **(see Figure 13 and Table 5)**. Hence, we use the two-sided paired t-test to compare the impact of the presence of the SiO_2 on the D_{50}. The significance level was set on 0.05 **(Table 6)**.

The paired t-test result (see below) **Table 7** shows that there is a significant difference between D_{50} values between granulates with and without the SiO_2. The addition of the SiO_2 apparently significantly shifts the PSD to the right (particles are larger).

Differences between reference lots in 20% ethanol in 0.1 M HCl media

The comparison of reference lots in 20 % ethanol in 0.1 M HCl media was performed using f_2 statistics on their dissolution

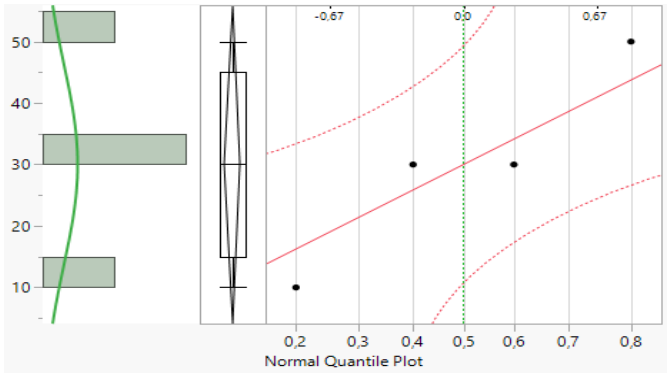


Figure 13 Distribution, fitted normal density curve and Q-Q plot of the difference of $D_{50}(SiO_2\text{-}noSiO_2)$.

Table 5. Anderson-Darling normality test for the difference of $D_{50}(SiO_2\text{-}noSiO_2)$.

	A2	Prob > A2
Anderson-Darling	0,2838914	0,5050

Table 6. Summary statistics of the difference of $D_{50}(SiO_2\text{-}noSiO_2)$.

Mean	30
Std Dev	16,329932
Std Err Mean	8,1649658
Upper 95% Mean	55,984565
Lower 95% Mean	4,0154347
N	4

profiles. The dissolution profiles of two lots are considered equivalent if the f_2 statistics exceeds 50. The dissolution profiles will be treated as significantly different if the 90 % confidence interval (CI) of the f_2 lies below the value 50 **(Table 8).**

For the purpose of the study, the 90% CI of f_2 was calculated using the bootstrap technique with 10.000 samples. The bias-corrected, accelerated CI type was used.

According to the **Table 9** the Lot 6 is significantly different from all other lots and the lot 5 is a borderline case regarding the comparison to lot 1 and to lot 2.

Table 7. Paired t-test for $D_{50}(SiO_2\text{-}noSiO_2)$.

Hypothesized Value	0
Actual Estimate	30
DF	3
Std Dev	16,3299
Test Statistic	3,6742
Prob > |t|	0,0349*
Prob > t	0,0174*
Prob < t	0,9826

Table 8. f_2 values.

	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
Lot 1	89.99	45.63	57.06	42.90	25.12
Lot 2		46.59	58.27	43.78	25.54
Lot 3			63.18	84.99	35.17
Lot 4				57.63	30.04
Lot 5					36.81

Table 9. BCa f_2 90% confidence intervals.

	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
Lot 1	(75.30, 93.65)	(28.01, 77.43)	(49.61, 63.38)	(37.80, 51.32)	(21.46, 28.85)
Lot 2		(28.78, 77.69)	(51.94, 62.93)	(39.30, 52.22)	(21.96, 29.16)
Lot 3			(39.62, 93.55)	(77.12, 96.92)	(24.97, 54.89)
Lot 4				(49.83, 71.98)	(25.64, 34.60)
Lot 5					(30.55, 44.30)

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