Dissolution Technologies

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Volume 31, Issue 1 | February 2024

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The scope of articles is limited to dissolution or disintegration topics as the major focus. Articles on formulation development where dissolution is just one test of many should not be submitted.

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The **May 2024** issue will include the USP Guideline on procedures for mechanical qualification and performance verification test Apparatus 1 and 2 (Tool Kit), and research articles on salicylic acid tablets with apparatus 4, selection of dissolution media, apparatus 2 inner diameter, amlodipine besylate tablets, and enalapril maleate tablets. The issue will also include AAPS 2023 annual meeting highlights and the Q and A feature.

Research Editor Vivian Gray vagray@rcn.com

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CORRESPONDENCE: Send all correspondence to: *Dissolution Technologies*, Inc., 9 Yorkridge Trail, Hockession, DE 19707. Phone (302) 235-0621, e-mail: vagray@rcn.com. Website: <u>www.dissolutiontech.com</u> (SSL certified).

STAFF: Founder, Cynthia Brown; Managing Director, Vivian Gray; Associate Editor, Valerie Clark; Research Editor, Vivian Gray; Research Editor, William Brown; Communications, Michael Larson; Circulation Manager, Sandra Larson; Layout, Michele Arnold; Publication, Printing, and Distribution Services, Archer Print Group, Sellersville, Pennsylvania.

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Advances in Product Quality and Performance Tests for Topical and Transdermal Products: View of the USP Expert Panel

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ABSTRACT

Quality and performance testing of topical and transdermal products encompasses a broad set of product types, test equipment, and unique considerations. This *Stimuli* article is one in a series of such articles on product testing methods that explore the relevant considerations and identify opportunities for standardization with different types of quality and performance tests. The objective of this *Stimuli* article is to highlight current knowledge gaps and potential challenges associated with quality and performance tests for certain topical and transdermal products, and to stimulate public input from product testing labs, product developers, regulators, and others. The input received may inform the development or revision of USP general chapters.

INTRODUCTION

uality and performance testing is a key part of formulation and product development. Appropriately developed test methods can facilitate an enhanced understanding of a product, and of the manner in which manufacturing process parameters alter the attributes and behavior of that product. These insights can help product developers mitigate the risks associated with inconsistent performance or unexpected failures during clinical development and manufacturing.

For topical and transdermal products, established performance tests described in the USP general chapter *Semisolid Drug ProductsPerformance Tests <1724>* (1) are routinely utilized to evaluate the rate of drug release, using an in vitro release test (IVRT), and the rate and extent of drug permeation into and through the skin, using an in vitro permeation test (IVPT). Best practices have been established for the development, validation, conduct, and analysis of IVRT and IVPT methods, and as a result, these tests are routinely used to guide the formulation, reformulation, process development, and control of topical semisolid dosage forms.

A detailed discussion of IVRT or IVPT methods, which are already well established, is beyond the scope of this article. Readers are referred to the following resources where the IVRT and IVPT methods are discussed in detail:

- Proceedings from the public workshop cosponsored by the US Food and Drug Administration and the Center for Research on Complex Generics, titled: In Vitro Release Test (IVRT) and In Vitro Permeation Test (IVPT) Methods: Best Practices and Scientific Considerations for ANDA Submissions" available at complexgenerics.org/IVRTIVPT (2).
- Proposed revision of <1724> in *PF* 48 (3). The proposed revision discusses the experimental design and method development considerations for IVRT and IVPT methods. Also, appropriate contexts for use of IVRT and IVPT studies are discussed, providing a guide for selecting which test method is appropriate based on the goals of the study.
- FDA Draft Guidance for Industry: In Vitro

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Permeation Test Studies for Topical Drug Products Submitted in ANDAs (October 2022) (3)

• FDA Draft Guidance for Industry: *In Vitro Release Tests Studies for Topical Drug Products Submitted in ANDAs* (October 2022) (4)

This Stimuli article focuses on novel dosage forms that utilize microneedles and novel product quality tests such as those which characterize the arrangement of matter in dosage forms. The development and assessment of these topical and transdermal dosage forms necessitates the identification and standardization of suitable practices, technologies, equipment, test methods, and data analysis procedures. In addition, certain existing test methods may have limitations, and may benefit from these improvements. This Stimuli article discusses current challenges and opportunities related to quality and performance testing in these areas, with the intent to stimulate public comments about how USP can contribute to the establishment of best practices and standards for such tests. This Stimuli article will specifically focus on the following novel product quality and performance test considerations for topical and transdermal dosage forms:

- In vitro adhesion tests for transdermal and topical delivery systems (collectively called TDS)
- In vitro quality and performance tests for microneedle array systems
- Physicochemical and structural (Q3) characterization tests for topical drug products

IN VITRO TDS ADHESION PERFORMANCE TESTS

The surface area of a TDS that is dosed upon the skin and remains adhered to the skin can modulate the amount of drug delivered into, and through the skin at any point in time. The entire contact surface area of a TDS should ideally remain consistently and uniformly adhered to the patient's skin throughout the duration of wear. When a TDS loses its adherence during wear, the amount of drug delivered to the patient may be reduced. Therefore, the adhesive properties and adhesion performance of a TDS product is routinely evaluated with tests that assess peel adhesion, release liner peel, and tack, as outlined within *Topical and Transdermal Drug Products-Product Quality Tests* <3> (5).

Each of these tests measures the force required to separate the TDS from another surface. In addition to characterizing the adhesive properties, cold flow and shear tests also measure the cohesive properties of a TDS formulation based on the resistance to flow of the adhesive matrix. Although useful to monitor batch-tobatch consistency, these tests have limitations, that make it challenging to correlate the test results with the in vivo adhesion performance of TDS. Thus, it is difficult to assess whether or not variations in manufacturing parameters that alter the results of these tests might also impact the clinical performance of the product.

A fundamental issue is that the current compendia! methods to evaluate the adhesive properties and adhesion performance of a TDS product are not designed to be biorelevant. However, such tests could be designed in a manner that systematically consider the influence on adhesion performance of intrinsic TDS attributes such as size, shape, adhesive type, adhesive system, adhesive formulation, TDS design, and the flexibility, stretchability, and occlusivity of the backing membrane. In addition, to help provide test results that relate to the real-world performance of a TDS on a patient, the tests may need to emulate extrinsic factors that have the potential to impact TDS adhesion, including the anatomically relevant temperature, curvature, torsion/flexion, softness, microtopographical features, moisture, and flaking (microdelamination) of the surface substrate.

Therefore, public input is sought from investigators who work with TDS products to clarify what intrinsic properties and extrinsic factors are most likely to influence in vivo adhesion performance, and to conceive novel test methods that are intentionally designed to monitor the performance of TDS products under biorelevant conditions. It may also be important to assess adhesion performance over time scales that are relevant to the wear period of the product, because the surface area of a TDS may progressively detach to greater degrees at longer time points. Also, in order to correlate in vitro adhesion test results with in vivo observations, it would be important to harmonize in vitro and in vivo study designs and control parameters.

PRODUCT PERFORMANCE TESTS FOR MICRONEEDLE ARRAY SYSTEMS

There are a variety of microneedle array systems being studied and/or under development, and there is a need for an in vitro performance test that would correlate with and be predictive of the in vivo performance of these products. Each microneedle variant may have unique aspects to characterize, whether it involves drug coated microneedles, dissolvable microneedles with drug formulated into the microneedles themselves, or larger capacity hydrogel dissolvable microneedles (Avcil et al.,

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2021) (6). A focus on the commonalities of the variants and the fundamental drug product attributes that are critical for microneedle drug product performance can help guide the development of a predictive in vitro performance test. There are two fundamental aspects relevant to clinical performance that are common to all microneedle variants: 1) microneedle insertion, and 2) dissolution/drug release of the active ingredient.

The product quality attributes and related considerations that may impact product performance (e.g., microneedle insertion) can include, but are not limited to, microneedle geometry (including length and spacing), tip sharpness, application velocity, force, and duration, as well as the impact of drug loading on microneedle strength for both coated and dissolving microneedles. Some of the mechanical testing related to microneedle insertion has been discussed by Lutton et al., 2015 (7); however, the most important aspect of product performance is to measure the microneedle penetration and the deposition of drug below the stratum corneum when a clinically relevant application force is used.

In relation to drug release/deposition below the stratum corneum, several product quality attributes can impact the product performance, such as the solubility of the drug, the formulation, the location of the drug in or on the microneedles, and the uniformity across the microneedle array with regard to location and duration of insertion. While the duration of microneedle application evaluated in many pre-clinical microneedle studies can be up to 24 h, it is preferable to minimize the application time in the clinical setting. An ideal performance test would ideally also identify a target duration of application (as well as a minimum time and maximum time, to guide human factors studies) that would provide consistent clinical performance while minimizing the application time.

Examples of performance test methods for a microneedle array system that combines assessments of microneedle insertion and in vitro drug release have been described by Larrañeta et al., 2015 (8). In one implementation of this methodology, dissolving microneedle arrays containing 196 needles (600 mm needle height) were inserted into a single layer of Parafilm M (PF), and a hermetic "pouch" was created including the array inside (Fig. 1A). The hermetic "pouch" containing the microneedle array system was placed in a dissolution bath and the rate of drug release was evaluated (Fig. 1B). Different microneedle formulations were tested using this methodology, releasing between 40 and 180 mg of a drug after 6 h. In another implementation of this methodology, the microneedle penetration through a PF membrane Dissolution Technologies | FEBRUARY 2024

was tested using a vertical diffusion cell (Fig. 1C) yielding comparable release curves. Microscopy was used in order to characterize the insertion of the different microneedle arrays in the PF membrane.



Figure 1. (A) Diagram illustrating the insertion and preparation of a microneedle pierced through a PF membrane and enclosed in a hermetic "pouch". (B) Diagram illustrating an in vitro release test in which the hermetic "pouch" is immersed in a dissolution vessel. (C) Diagram illustrating an in vitro release test in which the microneedle array system penetrates through a PF membrane mounted in a vertical diffusion cell. [Image courtesy of Larrañeta et al., 2015, (8)].

The performance tests described by Larrañeta et al., 2015 (8) illustrate how interdependent performance attributes may need to be considered in the design of suitable test methods. Ideally, pre-clinical and/or clinical data should be used as a basis for validating the test in order to assess an in vitro-in vivo correlation (IVIVC). For example, Tekko et al., 2022 (9) conducted a preclinical in vivo study using Sprague Dawley rats to evaluate a microneedle array system containing cabotegravir. Examples of such clinical studies with microneedles are limited. However, there is clinical data as well as pre-clinical data available for abaloparatide, including different formulations of coated microneedles (Bahar et al., 2015 (10); Hattersley et al., 2017 (11); Miller et al., 2021 (12). Such information could potentially provide a basis to validate a testing approach using an appropriate application force, velocity, and duration, as well as to provide additional validation to assess whether the aforementioned PF membrane (or another membrane that may serve as a mechanical surrogate for human skin) has the appropriate thickness, resistance to penetration, and elasticity to suitably represent how human skin influences the clinical performance of microneedle array systems. For example, the clinical performance of a microneedle array system may be influenced by the coating on the solid microneedles; if the drug was predominantly coated on regions close to the baseplate that do not penetrate below the stratum corneum to deliver the full dose, or if the coating on the needles had a tendency to be physically displaced from the tip to the baseplate upon microneedle insertion, then the drug delivery may be significantly impacted. An in vitro performance test that could discern such effects would be ideal.

The development of an in vitro performance test for microneedle array systems would ideally include optimization of the membrane that mechanically emulates relevant attributes of human skin, potentially leveraging ideas from performance tests developed for other complex dosage forms, such as the use of hydrophobized alginate hydrogels for the vesselsimulating flow-through cell described by Semmling et al., 2013 (13) for biorelevant drug-eluting stent testing. Alternatively, if the human skin is determined to be the optimal membrane to utilize in the test, it may be appropriate to evaluate whether a standardized test system may be utilized, such as commercially available preparations of ex vivo human skin in transwell systems described by Larson et al., 2021 (14) and developing a microneedle testing system based on such a model. One potential advantage of such a test system with viable human skin, is that it may also be able to assess certain skin responses to the application of a microneedle array system, and possibly the rate and extent of drug permeation through the deeper epidermal and dermal layers of the skin.

Therefore, public input is sought from investigators who work with microneedle array systems to comment on the current needs and uses for in vitro quality and performance test methods for these dosage forms, particularly relating to microneedle insertion performance testing and in vitro release testing. It would be helpful to receive comments relating to any considerations that may be unique to different types of microneedle array systems (coated, dissolvable, etc.) and to receive comments on the potential development of any preferred test system or testing methods currently utilized or proposed, including but not limited to those described above, which should be further developed to establish as a new USP compendia! test.

PHYSICOCHEMICAL AND STRUCTURAL (Q3) CHARACTERIZATION TESTS

When considering the critical quality attributes that modulate the performance of most liquid-based and other semisolid dosage forms (e.g., topical lotions, transdermal gels, vaginal creams), it is helpful to think about these within a conceptual framework that describes the type, amount, and arrangement of matter in the dosage form. The type of matter in a dosage form is routinely described by its ingredients, typically specified further in terms of a particular grade of that ingredient-this is a description of its qualitative components (i.e., Q1). The amount of each type of matter in a dosage form is routinely described by a formula that defines the relative proportion of each of the ingredients in the formulation-this is a description of its quantitative composition (i.e., Q2). Every batch of a pharmaceutical product is designed to have the same Q1 and Q2 attributes (within specified tolerances) because significant differences in the components or composition of a product may alter its performance from batch to batch.

In addition, manufacturing process parameters are also controlled within specified limits, because they can influence the arrangement of matter in the dosage form. This is very important, because the resulting physicochemical and structural (Q3) attributes are analogous to the molecular machinery within a dosage form that modulates numerous aspects of its performance. Thus, ensuring consistency in the Q1, Q2, and Q3 attributes of a product helps ensure consistent product performance. Regulatory concepts relating to Q3 characterization are described in FDA's Draft Guidance for *Physicochemical and Structural (Q3) Characterization of Topical Drug Products (15)*.

There are established compendial standards to characterize the type, grade, and purity/potency of many ingredients which are routinely utilized in topical and transdermal products, so describing the Q1 and Q2 attributes of a product is relatively straightforward. Characterizing the Q3 attributes of a product typically involves a collection of specific tests that individually describe specific product attributes, and collectively describe the arrangement of matter in ways that are useful. However, different test methods can sometimes be used to characterize a particular Q3 attribute of a product, and the different methods may not provide the same information, so identifying and optimizing appropriate, standardized test methods for Q3 characterization is exceptionally useful.

Perhaps the simplest of the Q3 tests characterizes the appearance and texture of a product and may also describe attributes like odor. This test is frequently performed using human sensory assessments that describe the look and feel of a product as well as its smell, if relevant. Microscopic examination of the product can

help to characterize the number and type of phase states, describing features like globules and suspended particles. This can help to characterize the structural organization of matter in the dosage form, potentially defining whether it is an emulsion, what the globule size distribution or particle size distribution is, as well as identifying features like polymer matrices or crystal habits of any suspended drug.

These tests help us to understand the architecture and potential interactions among the molecular machinery of the system. For example, differences in globule size distributions would correspond to various factors such as differences in the surface area across which dissolved drug may partition from the globules to the continuous phase, and differences in the proportion of total interfacial surface area with the skin that may be occupied by the cross section of a globule, from which drug partitioning from a globule into the skin may be different than the same drug partitioning from the continuous phase into the skin.

The Q3 characterization of topical dosage forms is particularly important because their physicochemical and structural features may not be evident from their dosage form nomenclature. For example, a lotion may actually be a viscous single-phase solution, a gel may be an emulsion, a cream may not have globules, an ointment may or may not contain any petrolatum, and any of these may contain fully dissolved or partially suspended drug. If there is suspended drug, it would be appropriate to characterize the polymorph(s), and to characterize them within the drug product. If different polymorphic forms of the drug exist, then the control of these polymorphs in the product may be determined based on considerations outlined in decision tree #4 within the International Council for Harmonisation (ICH) specifications (*16*).

It is particularly important to recognize that the performance of topical and transdermal dosage forms may be modulated by their metamorphosis following their application on the skin, and potentially even by the metamorphosis during product dispense and dose administration. For example, many semisolid dosage forms are shear thinning, and differences in apparent viscosity may have the potential to alter the drug diffusion within the dosage form, flow properties on the microtopography and into the appendages of the skin, retention at the site of application, transfer to an unintended recipient, and other considerations.

The rheological behavior of a product reflects how the components interact within the molecular machinery,

and how the system responds to stress. This typically involves using a rheometer that is appropriate for monitoring the potentially non-Newtonian flow behavior of liquid and semisolid dosage forms. Whenever it is feasible, it is ideal to characterize the flow curves across a range of attainable shear rates, typically until low- or high-shear plateaus are identified; at a minimum, it is important to characterize the apparent viscosity at low-, medium-, and high-shear rates. The best way to visualize comparative rheology data for a test and reference product is by plotting the data for both, shear stress versus shear rate, and viscosity versus shear rate. Also, if the product exhibits plastic flow behavior, then the yield stress should be characterized, and if it is relevant, the linear viscoelastic response can also be very informative; a good way to visualize this, is by plotting the storage and loss moduli versus frequency.

Another phenomenon that occurs during the metamorphosis of topical and transdermal dosage forms is evaporation of volatile components, including water. As these components evaporate, the composition of the product formulation changes, and this can lead to changes in drug solubility that alter the drug concentration as well as the amount of dissolved drug available for partitioning into the skin, along with alterations in the thermodynamic activity of the drug in the product (residue) on the skin. Therefore, it may be important to characterize the solvent (water) activity using an appropriate device, or to measure the drying rate gravimetrically, at relevant temperatures.

It is well understood that physicochemical properties like the pH of a product can have a substantial impact on a variety of potentially critical quality attributes, such as the viscosity of a gel or the ionization state of the drug, and the pH of the product following application upon the skin may be dependent upon how well the product is buffered. So, it may also be important to characterize the pH of products with aqueous formulations, and to characterize any buffer systems as well.

By contrast, for products comprised of more than 70% oleaginous contents (like many petrolatum-based ointments), it is typically feasible to characterize the product using the tests listed in the *USP* monograph for petrolatum, recording quantitative test results such as the actual pH of the pooled washings during an alkalinity test with a calibrated pH meter, or recording the result for a drop point test (described in *USP* general chapter <741>) as the average observed melting temperature. In addition to the quantitative tests, qualitative characterization of the relative proportions of different hydrocarbons

in petrolatum-based ointments may be particularly important since petrolatum is comprised of a mixture of hydrocarbon species, and differences in the proportions of hydrocarbons in that mixture may also alter the drug delivery from the ointment to the skin.

Different manufacturing process parameters (e.g., mixing rate and duration) may have the potential to alter the amount of entrapped air in a product formulation, which may in turn impact the delivered dose, so it may be considered prudent to characterize the specific gravity of a topical or transdermal semisolid product. Also, different packaging configurations may influence the shear forces exerted on the dosage form during dispensing (e.g., from a tube vs. a pump), so it may be important to characterize the influence of the container closure system on the Q3 attributes of the dispensed product. Additionally, product metamorphosis may occur as a function of aging, so it may be important to characterize Q3 attributes at different points in time across the shelf life of the product; the corollary is that, when characterizing multiple batches of a product, it is prudent to monitor trends where Q3 attributes may progressively change as a function of age.

Suffice it to say that characterization of the Q3 attributes of a topical or transdermal dosage form can be exceptionally informative, because these attributes can modulate how the product will perform under clinical use conditions, and because these Q3 characteristics enable us to systematically compare different aspects of the arrangement of matter between batch to batch of a product, between a reference and test batch of a product that may experience a post-approval change, and between a reference standard product and a generic product. The challenge is that compendia! test methods do not yet exist for many of the tests that may be utilized to facilitate Q3 characterization. For example, there are different methods, equipment, and test conditions that may be utilized to characterize Q3 attributes as simple as pH, or as complex as rheological behavior. Particle size distribution may be characterized in the dosage form by using optical microscopy or morphologically directed Raman spectroscopy. Similarly, the polymorphic form(s) of suspended drug in the dosage form may be characterized by X-Ray diffraction or by Raman spectroscopy.

Therefore, public input is sought from investigators who work with topical and transdermal products to clarify whether it is challenging to identify appropriate test methods, equipment, and conditions, and to determine the appropriate number of replicate measurements or the relevant data analysis and reporting considerations. It would be exceptionally valuable to ascertain from public comments to this *Stimuli* article whether USP should establish compendia! tests that represent a comprehensive tool kit of methods that can be utilized for Q3 characterizations of topical and transdermal products.

CONCLUSION

This article was written to raise awareness of the diversity and challenges to develop product performance tests methods for topical and transdermal drug products. It is the authors hope that the topics noted in this article will stimulate collaborative and harmonized research to develop test methodologies to become standards which can be incorporated into future compendia! chapters.

DISCLAIMER

The views presented in this article do not necessarily reflect those of the FDA. No official support or endorsement by the Food and Drug Administration is intended or should be inferred.

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Logan Fully Automated Dissolution System



This system precisely delivers, preheats, and degasses media into 8 dry-heat vessels removing the added complication of a water bath. Each vessel has bottom and side cameras to record the dissolution for subsequent viewing and result verification. After the test is over, the vessels automatically empty the media, they are then sprayed, washed, and blow-dried, ready for the next 9 batches of test samples.

Logan System 2400 is equipped with two types of filter changers. The Super 2400 dissolution tester includes an automated filter tip changer for online UV analysis. An additional membrane filter changer is available for sample collection for offline HPLC analysis.



Online UV analysis



Filter changer for collection of samples for offline HPLC analysis



Logan PVT 800 toolset performs and records dissolution Performance Validation Testing (PVT) electronically, removing the uncertainties of manual recording. Logan PVT 800 toolset includes a temperature sensor, speed detector, vertical/horizontal, height, vibration, and centering gauges, etc. The PVT report is displayed on the PC screen, the data can be recorded in the hard drive and printed. With this advanced toolset, user intervention in the data-transferring process is eliminated. Logan PVT 800 toolset further improves compliance with FDA CFR 21 part 11.

An In Vitro Model for Release of Acetaminophen When an Overdose is Ingested Orally

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ABSTRACT

Acetaminophen (paracetamol) is an analgesic and antipyretic drug that is widely used across the globe due to its efficacy and safety within the therapeutic dose range. When acetaminophen is ingested in amounts higher than the recommended dosage over several days, severe hepatotoxic effects can result. Next to the opioid drugs, acetaminophen is often deliberately ingested as an overdose, in which case multiple dosage units may be ingested. In this study, a novel in vitro model aiming to understand the dissolution of acetaminophen in overdose situations is described and implemented to compare four commercially available formulations of acetaminophen. Increasing quantities of immediate-release and extended-release tablets as well as hard and soft capsule formulations were tested in the in vitro model using United States Pharmacopeia apparatus 3 (reciprocating cylinder). When a single dose was tested, acetaminophen dissolved rapidly from the immediate-release formulation and from the immediate release part of the extended-release formulation. At higher doses, acetaminophen was released more slowly and less extensively as the dose was increased. The results obtained with the in vitro model are in line with the literature data obtained in acetaminophen clinical trials. Additionally, the in vitro model was able to reproduce pharmacobezoar formation at very high doses, which has been observed in cases of deliberate overdose.

KEYWORDS: acetaminophen, paracetamol, overdose, dissolution, USP apparatus 3

INTRODUCTION

cetaminophen (also known as paracetamol) is an analgesic and antipyretic drug, belonging to Biopharmaceutical Classification System class 3 (highly soluble, low permeability) (1). Acetaminophen was introduced in the early 1960s as an alternative to acetylsalicylic acid (aspirin), having lesser side effects, e.g. in the gastrointestinal tract. First popular in Europe, it gained popularity in the USA in the 1980s after Reyes syndrome in children was linked to aspirin (2). It is currently marketed throughout the world as tablets, capsules, syrups, or suspensions for oral administration, as well suppositories for rectal application.

After administration within its therapeutic dose range (recent recommendations are max 4 g daily), it is absorbed well, and its peak concentration in serum is observed after about an hour (3-5). Therapeutic doses are well tolerated by most patients and are safe in comparison to most non-steroid anti-inflammatory drugs. A few years after acetaminophen was marketed for the first time, it was reported that severe adverse effects could

occur, especially concerning the liver (2). Nowadays, it is well known that in doses only slightly higher than recommended (so-called unintentional overdoses), acetaminophen ingestion may cause severe hepatotoxic effects within a few days and can lead to acute liver failure (2). On the other hand, much higher overdoses of acetaminophen (single doses even above 200 g) may be ingested deliberately in a suicidal attempt (2, 5, 6). Irrespective of whether the overdose is intentional or not, such cases present a severe burden to both patients and the public health care system in many countries. Several clinical trials of immediate-release (IR) dosage forms of acetaminophen in healthy volunteers, up to 2 g of a single dose, have been published (5, 7). Yet, there is still a lack of in vitro studies aimed at understanding the dissolution of acetaminophen at these and higher levels of overdose.

The current study presents a novel in vitro dissolution method, based on United States Pharmacopeia (USP) apparatus 3 (reciprocating cylinder), for comparing various marketed acetaminophen oral dosage forms after overdose. This method is applied to IR tablets, extended (modified) release (ER) tablets, soft capsules, and hard capsules.

METHODS

Chemicals

The following acetaminophen (paracetamol) formulations were chosen for study: IR tablets (paracetamol STADA 500 mg, lot 14814, STADA Arzneimittel AG, Bad Vilbel, Germany), hard capsules (ben-u-ron 500 mg, lot 703M211, bene-Arzneimittel GmbH, Munich, Germany), soft capsules (APAP_{caps} 500 mg, lot U1110771, US Pharmacia Sp. z o.o., Wroclaw, Poland), and modified release bi-layer (ER) tablets (Osteomol 665 mg, lot 22121357, Pharmacor Pty Ltd., Chatswood, Australia). All products were purchased commercially.

Hydrochloric acid 1 M solution (VWR Chemicals, lot 22C024019), maleicacid (Merck, lot S7667380943), sodium chloride (Carl Roth, lot 076235484), sodium hydroxide pellets (VWR Prolabo, lot 14A100027), sodium phosphate monobasic dihydrate (Merck, lot K91237142611), and tris base (Sigma-Aldrich, lot SLBP4240V) were used for preparation of the media buffers.

Acetonitrile, methanol, and trifluoroacetic acid (TFA), all analytical grade, were purchased from VWR International (Darmstadt, Germany). Paracetamol Chemical Reference Standard (CRS, Eur. Pharm., Batch 4.1), used as an analytical standard, was also purchased from VWR International.

Acetaminophen Solubility

The solubility of acetaminophen in the presence of excipients present in the IR tablets was tested in the buffers on which fasted state simulated gastric fluid (FaSSGF), fasted state simulated intestinal fluid version 2 (FaSSIF-V2), FaSSIF at midgut (FaSSIF_{midgut}), simulated intestinal fluid in the ileum (SIF_{ileum}), and fed state simulated colonic fluid (FeSSCoF) are based. The solubility experiments were performed in triplicate using a multiposition magnetic stirrer assembly (Variomag Poly 15, H+P Labortechnik GmbH, Oberschleißheim, Germany). The IR tablets were crushed and pulverized using a mortar and pestle. An excess of pulverized formulation (186 mg of powder, corresponding to 150 mg of acetaminophen) was weighed using an analytical balance (SECURA 225-D-1S, Sartorius AG, Göttingen, Germany) and transferred into a 20-mL glass container with a screw cap. A 15-mm stirring bar was placed into the vial, and 5 mL of media buffer was added. Subsequently, the vial was placed on the magnetic stirrer in a preheated incubator (IncuLine 68R, VWR International, Leuven, Belgium). The samples

were incubated at 37 ± 1 °C for 24 hrs under stirring at 600 rpm. Samples (1.2 mL) were withdrawn after 24 hrs of incubation using 2 mL syringes (Injekt Luer Solo, lot 21G08C8, B.Braun, Melsungen, Germany) equipped with 21 G x 1.5-in. needles (FINE-JECT, lot 14-12300, HENKE SASS WOLF, Tuttlingen, Germany). The samples were filtered through 0.45-µm polytetrafluoroethylene (PTFE) membrane syringe filters (Acrodisc, LOT FC5752, PALL Corporation, Port Washington, NY, USA), discarding the first 0.8 mL and collecting the last 0.4 mL of filtrate for high-performance liquid chromatography (HPLC) analysis. HPLC analysis was performed after the samples were appropriately diluted with mobile phase.

Dissolution Testing

USP Apparatus 2 with Low Volume

In preliminary experiments, the dissolution of acetaminophen from the IR tablets was tested in a USP apparatus 2 (PTDT 820D, Pharma Test Apparatebau AG, Hainburg, Germany) equipped with scaled-down vessels (250 mL) and paddles. These experiments were performed in triplicate.

Doses of 1 or 10 acetaminophen IR tablets (500 mg and 5000 mg/vessel, respectively) were added to 100 mL of FaSSGF buffer or FaSSIF version 1 (FaSSIF-V1) buffer. The experiments in FaSSGF buffer were conducted for 2 hours and those in FaSSIF-V1 buffer for 6 hours. During the experiments, a temperature of 37 ± 0.5 °C was maintained.

Samples (5 mL) were withdrawn at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min (FaSSGF buffer) and at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min, and hourly thereafter (FaSSIF-V1 buffer) using a set of sampling cannulas equipped with Poroplast 10-µm cannula filters (ERWEKA, Langen, Germany) and 5-mL Omnifix Luer Lock Solo syringes (lot 22C21C8, B.Braun, Melsungen, Germany). The samples were filtered through 0.45-µm PTFE membrane syringe filters (Whatman Puradisc, lot A29640934, Cytiva, Marlborough, MA, USA), returning the first 4 mL of filtrate back into the vessel and collecting the last 1 mL for analysis. Collected samples were analyzed by HPLC after appropriate dilution with the mobile phase. After each sample was withdrawn, the media in the vessels was replenished by adding 1 mL of fresh, preheated medium.

To determine whether further dissolution would occur in more distal regions of the GI tract, it was necessary to switch to an apparatus that facilitates multiple media changes. For this reason, USP apparatus 3 was selected for further experiments.

USP Apparatus 3

The four acetaminophen formulations (i.e., IR tablets, ER tablets, hard capsules, soft capsules) were subjected to dissolution testing using USP apparatus 3 (reciprocating cylinder) (RRT 10 Caleva, ERWEKA, Langen, Germany). The dissolution tester was equipped with 250-mL flatbottom vessels and inner glass cylinders fitted with two 420-nm nylon meshes, one at the upper and one at the lower cylinder opening. All experiments in USP apparatus 3 were performed in triplicate.

The dissolution media used for this assembly were the buffers contained in FaSSGF, FaSSIF-V2, FaSSIF_{midgut}, SIF_{ileum}, and FeSSCoF according to Markopoulos et al. (8). The compositions of these buffers are presented in Table 1. The vessels, each containing 220 mL of medium, were placed in a water bath in order of their sequence in the gastrointestinal (GI) tract, and a temperature of 37 ± 0.5 °C was maintained during the experiment. During the experiment, motility of the human GI tract was simulated by changing the dipping rate of the inner cylinders, as shown in Figure 1 and described in Table 2.

All formulations were tested for acetaminophen dissolution from single doses (500 mg for IR tablets and hard and soft capsules; 665 mg for ER tablets) and from two levels of overdosing: 1) 10 tablets or capsules per

vessel (total dose of 5 g for IR and 6.65 g for ER dosage forms) and 2) 20 tablets or capsules per vessel (total dose of 10 g for IR and 13.3 g for ER dosage forms). Additionally, the IR tablets were tested for acetaminophen dissolution from 50 tablets per vessel, corresponding to a total dose of 25 g of acetaminophen.





Samples (5 mL) were withdrawn after 10 and 20 min from the FaSSGF buffer and every 20 minutes thereafter from the FaSSGF, FaSSIF-V2, and FaSSIF_{midgut} buffers. When

Components	FaSSGF	FaSSIF-V1	FaSSIF-V2	FaSSIF _{midgut}	SIF _{ileum}	FeSSCoF
Tris base (mM)						30.5
Maleic acid (mM)			19.1	19.3	52.8	30.15
Sodium hydroxide (mM)		13.8	34.8	36.5	105	16.5
Sodium chloride (mM)	34.2	105.8	68.6	76.1	30.1	34.0
Sodium phosphate monobasic (mM)		28.7				
Hydrochloric acid	q.s. pH 1.6					
рН	1.6	6.5	6.5	6.8	7.5	6.0
Dissolution apparatus	USP 2 & 3	USP 2	USP 3	USP 3	USP 3	USP 3

*Buffers only (no bile components added).

FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid; SIF: simulated intestinal fluid; FeSSCoF: fed state simulated colonic fluid.

Table 2. USP Apparatus 3 Testing Setup and Media Details

	5 1				
Row number	Media buffer*	Buffer pH	Duration (min)	Cumulative duration (min)	Dip rate (dips/min)
1	FaSSGF	1.6	60	60	12
2	FaSSIF-V2	6.5	40	100	10
3	FaSSIF _{midgut}	6.8	80	180	10
4	SIF _{ileum}	7.5	60	240	10
5	FeSSCoF	6.0	120	360	6

*Buffers only (no bile components added).

FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid; SIF: simulated intestinal fluid; FeSSCoF: fed state simulated colonic fluid.

release in SIF_{ileum} and FeSSCoF buffers was tested, samples were withdrawn every 30 minutes. The samples were withdrawn using 5-mL Omnifix Luer Lock Solo syringes through sampling cannulas equipped with Poroplast 10- μ m cannula filters. The samples were subsequently filtered through PTFE 0.45- μ m membrane syringe filters, returning the first 4 mL of the filtrate into the vessel and collecting the last 1 mL for the HPLC analysis, which took place after appropriate sample dilution. In these experiments, the withdrawn media in the vessels was not replenished with fresh buffer after sampling, but the volume loss was accounted for when calculating the acetaminophen concentrations.

Analytical Method

Analysis of the samples collected in solubility and dissolution experiments was performed using reversed phase HPLC. The concentration of acetaminophen was measured using a Chromaster VWR/HITACHI HPLC system (VWR International) equipped with a 5110 pump, 5210 autosampler, 5310 column oven, 5410 UV-Detector, and Agilent OpenLab EZChrom software (version A.04.10).

The analytical method was based on the method of Franeta et al., adjusted to shorten the elution time for acetaminophen while still obtaining well-defined peaks (9). The separation was performed using a LiChrospher 100 RP18 endcapped 5-µm 250-4 analytical column (Merck Millipore, Darmstadt, Germany) as the stationary phase. The mobile phase consisted of acetonitrile (VWR International) and MilliQ water (in-house filtration, high purity water system Ultra Clean GP UV UF, EVOQUA Water Technologies LLC, Günzburg, Germany) in a ratio of 15:85 v/v, to which 0.05% TFA was added. A flow rate of 0.8 mL/min resulted in a retention time of 5.1 min. Peak detection was performed at 240 nm. The limit of quantification (LOQ) of the analytical method was 1.46 μ g/mL. For each sample set, a fresh calibration curve was prepared. The coefficient of determination (R^2) calculated for the calibration curves was always > 0.999.

Data Presentation and Statistical Analysis

Microsoft Excel (2016, Redmond, WA, USA) with the Data Analysis Tool Pack was used to calculate the acetaminophen concentration in each sample. For the statistical analysis of the data obtained during the experiments, SigmaPlot (version 12.5, SYSTAT Software Inc., Point Richmond, CA, USA) was used. All data are presented as mean values ± standard deviation. Oneway analysis of variance (ANOVA) and post-hoc pairwise multiple comparison procedures using the Holm-Sidak method were performed. The acetaminophen formulations were compared with each other at the

doses tested in the USP apparatus 3 assembly at the 10and 60-min time points, and alpha was set to 0.05.

For the statistical comparison of two dissolution acetaminophen profiles, the similarity factor f_2 was calculated (without the optional weighting factor). The similarity factor is a statistical tool described by FDA to determine the equivalence of dissolution profiles from oral dosage forms (10). If f_2 is 50 or greater, this indicates that the two compared dissolution profiles differ by 10% or less from each other. The f_2 factors for each acetaminophen dissolution profile pair using data from USP apparatus 3 experiments were calculated using the first four sampling points in FaSSGF buffer. For dissolution profiles with more than four sampling points, the f_2 factor was calculated using all available time points.

RESULTS

Solubility

The solubility results for acetaminophen in the IR tablets are presented in Table 3. The solubility values after 24 hrs of incubation were consistent across all media tested, ranging from 23.60 ± 0.32 mg/mL in FaSSIF_{midgut} buffer to 24.82 ± 0.96 mg/mL in FeSSCoF buffer.

Table 3. Solubility (mg/mL) of Pulverized Paracetamol STADA (acetaminophen) 500-mg immediate-release tablets in USP 3 Dissolution Media Buffers*

FaSSGF	FaSSIF-V2	FaSSIF midgut	SIF _{ileum}	FeSSCoF
23.98 ± 0.21	23.68 ± 0.95	23.60 ± 0.32	23.62 ± 0.31	24.82 ± 0.96

*Values are mean \pm SD (n = 3)

*Buffers only (no bile components added).

FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid; SIF: simulated intestinal fluid; FeSSCoF: fed state simulated colonic fluid.

USP Apparatus 2 Dissolution

When one dose or 10 doses were tested in USP apparatus 2 with scaled-down vessels and paddles, the IR tablets fully disintegrated within the first minute after contact with FaSSGF buffer or FaSSIF-V1 buffer. After 30 min, more than 80% of the dose (i.e., 89.3 \pm 1.6% in FaSSGF buffer and 82.9 \pm 5.0% in FaSSIF-V1 buffer) had been released from a single tablet (Fig. 2). The profiles reached a plateau after 45 min in FaSSGF buffer and after 60 min in FaSSIF-V1 buffer. A plateau concentration of 4.74 \pm 0.05 mg/mL (95.8 \pm 1.1% of the total dose) was measured at 90 min in FaSSGF buffer. In FaSSIF-V1 buffer, a plateau concentration of 4.77 \pm 0.05 mg/mL (96.4 \pm 1.0%) was measured at 360 min.

When 10 tablets were tested, the percent release was lower than that of one dose in both FaSSGF and FaSSIF-V1 buffers. Similar to the dissolution of one dose, the profiles reached a plateau after 45 min in FaSSGF buffer and after



Figure 2. Paracetamol (acetaminophen) 500-mg IR tablets dissolution profiles tested using a low volume USP apparatus 2 method. Profiles are for one dose (diamonds) and 10 doses (circles). Top row shows mean acetaminophen concentration and solubility (dashed line) measured in various biorelevant buffers over the range of pH 1.2–9 based on Shaw et al. (11). Bottom row shows mean drug release and maximum dissolvable % for 10 tablets (solid line). IR: immediate release; FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid.

60 min in FaSSIF-V1 buffer. In FaSSGF buffer, a plateau concentration of 20.68 \pm 0.22 mg/mL was reached by 2 hrs, which corresponds to 41.8 \pm 0.4% of the total dose. In FaSSIF-V1 buffer, the plateau concentration at 240 minutes was 20.71 \pm 0.34 mg/mL, which corresponds to 41.8 \pm 0.7% of the total dose. In these experiments, acetaminophen dissolution from 10 IR tablets is limited by its solubility.

USP Apparatus 3 Dissolution

Dissolution profiles from all tests in USP 3 apparatus are shown in Figure 3. Photographs of the dosage forms at various stages of the tests are shown in Figures 4–6.

Immediate-Release Tablets

Acetaminophen dissolved rapidly from one IR tablet, reaching $89.8 \pm 1.4\%$ release (449 \pm 7 mg) after 10 min in the first (FaSSGF buffer) compartment. After 60 min in

18 Dissolution Technologies FEBRUARY 2024 www.dissolutiontech.com FaSSGF buffer, some disintegrated tablet residues were still present in the inner cylinders. The experiment was therefore continued in the FaSSIF-V2 buffer compartment, where $100.9 \pm 0.9\%$ of the total dose was released ($504 \pm 4.5 \text{ mg}$) by 100 min. At this time, all tablet residues had passed through the bottom mesh into the outer vessel.

When 10 doses were tested, rapidly disintegrated IR tablets formed a dense clump (Fig. 4B.1), resulting in some disruption of the flow pattern inside the inner cylinder. The clumping slowed the acetaminophen dissolution profile in comparison to that of one tablet. At the last time point (60 min) in FaSSGF buffer, 72.0 \pm 7.7% of the total dose had been released. The experiment was therefore continued into the FaSSIF-V2 buffer, where 94.8 \pm 2.9% of the total dose was released, and then in FaSSIF_{midgut}, where the tablet residues penetrated through the nylon



Figure 3. Dissolution profiles of four acetaminophen formulations tested using USP apparatus 3, expressed as mean \pm SD percent of the tested dose (n = 3). Profiles show one dose (triangles), 10 (circles), 20 (diamonds), and 50 doses (squares, IR tablets only). FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid; SIF: simulated intestinal fluid; FeSSCoF: fed state simulated colonic fluid; IR: immediate release; ER: extended release. Note that in each case the buffer of the biorelevant medium was applied.

mesh into the outer vessel, releasing $97.3 \pm 2.8\%$ of the dose, corresponding to 4866 ± 140 mg of acetaminophen.

When 20 IR tablets were tested, they also disintegrated quickly (within the first 10 sec after contact with FaSSGF buffer), but then formed a dense clump (Fig. 5B.1), obstructing media flow between the inner and outer cylinders. After 60 min in FaSSGF buffer, only 18.5 \pm 0.2% of the total dose (1845 \pm 24 mg) was released. The experiment continued in the FaSSIF-V2, FaSSIF_{midgut}, and SIF_{ileum} buffer compartments, in which 33.3 \pm 2.7% (3335 \pm 265 mg), 53.7 \pm 6.8% (5374 \pm 684 mg), and 67.3 \pm 10.1% (6727 \pm 1007 mg) of the total acetaminophen dose was

released, respectively. Because some tablet residues were still present in the inner cylinder, testing in FeSSCoF buffer was also conducted. During this part of the experiment, the rest of the tablet material penetrated through the nylon mesh into the vessel. After 6 hrs of testing (in all five compartments), 20 IR tablets released $103.0 \pm 0.9\%$, corresponding to $10,300 \pm 87$ mg of acetaminophen.

When 50 IR tablets were tested, rapid tablet disintegration followed by clumping was again observed (Fig. 6). At the final time point of the FaSSGF buffer compartment, only $3.9 \pm 3.2\%$ of acetaminophen total dose had been released. In further compartments, the percent release

rose to $5.0 \pm 2.8\%$ in FaSSIF-V2, $7.8 \pm 2.2\%$ in FaSSIF_{midgut}, $8.9 \pm 2.2\%$ in SIF_{ileum}, and $10.8 \pm 2.2\%$ in FeSSCoF. This final percent release corresponds to 2695 \pm 547 mg released acetaminophen in total, considerably less than values for either 10 or 20 tablets.

Extended-Release Tablets

In all experiments, the outer layer of the ER tablets disintegrated shortly after coming in contact with FaSSGF buffer, and an initial dose of acetaminophen was released.

When one ER tablet was subjected to dissolution, $50.6 \pm 1.5\%$ (336 ± 10 mg) was released within 10 min in FaSSGF buffer. This corresponds to the IR fraction of the tablet. Subsequent acetaminophen dissolution was slower, reaching 67.3 ± 1.5% (447 ± 10 mg) at the end of the test period (60 min) in FaSSGF buffer. On the lower mesh of the inner cylinder, a moist and slightly swollen tablet core was observed after finishing the FaSSGF buffer part of the



Figure 4. Photographs of 10 doses of acetaminophen during USP apparatus 3 testing. Row 1 shows IR tablets, 2 shows ER tablets, 3 shows hard capsules, and 4 shows soft capsules. A.1–A.4 depicts the dosage forms in the inner cylinder shortly before test start; B.1–B.4 is within first 2 minutes in FaSSGF; C.1–C.3 is after 60 minutes in FaSSGF, C.4 shows 10 soft capsules after 15 minutes in FaSSGF; D.1–D.3 shows inner cylinder content after finishing the test, and D.4 shows strongly colored FaSSGF buffer due to penetration of all soft capsule residuals into the outer vessel. USP: United States Pharmacopiea; IR: immediate release; ER: extended release; FaSSGF: fasted state simulated gastric fluid.

test. This remnant persisted throughout the subsequent compartments, including FeSSCoF buffer. In FaSSIF-V2, release reached 73.7 \pm 2.2% (490 \pm 15 mg), 81.4 \pm 1.5% (542 \pm 10 mg) in FaSSIF_{midgut}, 86.0 \pm 1.3% (572 \pm 8 mg) in SIF_{ileum}, and 92.2 \pm 2.0% (613 \pm 13 mg) in FeSSCoF, in accord with the ER properties of the tablet.

When 10 doses were tested, the rapidly disintegrated outer layer of the ER tablets formed a dense clump of residual material inside the inner cylinder (Fig. 4B.2), which impeded media flow. After 60 min in FaSSGF buffer, $25.1 \pm 5.6\%$ (1671 ± 370 mg) of acetaminophen had been released. Subsequently, acetaminophen release reached $32.8 \pm 9.4\%$ (2179 ± 624 mg) in FaSSIF-V2, $40.9 \pm 13\%$ (2719 ± 862 mg) in FaSSIF_{midgut}, $44.7 \pm 13.5\%$ (2975 ± 897 mg) in SIF_{ileum}, and $60.7 \pm 12\%$ (4034 ± 796 mg) at the end of the test (6 hrs).

When 20 ER tablets were tested, disintegration of the



Figure 5. Photographs of 20 doses of acetaminophen during USP apparatus 3 testing. Row 1 shows IR tablets, 2 shows ER tablets, 3 shows hard capsules, and 4 shows soft capsules. A.1–A.4 depicts the dosage forms in the inner cylinder shortly before test start; B.1–B.4 is within first 2 minutes in FaSSGF; C.1–C.3 is after 60 minutes in FaSSGF, and C.4 is after 20 minutes in FaSSGF, with media present in the inner cylinder due to clumped capsule material on the bottom mesh; D.1–D.3 shows inner cylinder content after finishing the test, and D.4 shows strongly colored FaSSGF buffer due to penetration of all soft capsule residuals into outer vessels 1 and 3 (not observed in vessel 2).

IR: immediate release; ER: extended release; FaSSGF: fasted state simulatec gastric fluid

outer layer again caused obstruction of the media flow due to the dense clump of residual material (Fig. 5B.2). In FaSSGF buffer, release amounted to just 7.4 \pm 0.8% of the total dose (989 \pm 111 mg). The experiment continued through the FaSSIF-V2, FaSSIF_{midgut}, SIF_{ileum}, and FeSSCoF buffer compartments, where 8.8 \pm 0.7% (1172 \pm 98 mg), 15.0 \pm 4.8% (1998 \pm 641 mg), 18.7 \pm 7.0% (2487 \pm 928 mg), and 25.3 \pm 12.7% (3364 \pm 1691 mg) of the total acetaminophen dose was released, respectively. After the test was finished, some tablet residues were still present on the lower mesh of the inner cylinder (Fig. 5D.2).

Hard Capsules

The ben-u-ron gelatin capsule shells started to dissolve within 2 minutes after the test was started, releasing granular acetaminophen and talc. Single hard capsules released acetaminophen rapidly, reaching 83.1 \pm 1.0% release (416 \pm 5 mg) after 10 minutes in FaSSGF buffer. After 1 hr, 97.9 \pm 0.5% (489 \pm 2 mg) of acetaminophen had been released and no residual capsule material was present in the inner cylinders. Therefore, the experiment was not continued.

When 10 doses were tested, the residual material formed a clump after the capsule shells started to dissolve (Fig. 4C.3), impeding media flow. After 60 min, 57.7 \pm 7.9% (2884 \pm 393 mg) of the total dose had been released. The experiment continued in the FaSSIF-V2 buffer, where release reached 75.3 \pm 6.1% (3763 \pm 306 mg). In the FaSSIF_{midgut} buffer compartment, all capsule material residues penetrated through the nylon mesh into the outer vessel, reaching 76.9 \pm 6.5% drug release, which corresponds to 3846 \pm 343 mg of acetaminophen.

When 20 hard capsules were tested, the residual material again formed a dense clump after dissolution of the capsule shells (Fig. 5C.3). This caused obstruction of the media flow. After 60 min in FaSSGF buffer, only 22.9 \pm 1.3% (2292 \pm 134 mg) of the total dose had been released. The experiment was continued through the FaSSIF-V2, FaSSIF_{midgut}, and SIF_{ileum} buffers, releasing 31.2 \pm 3.6% (3116 \pm 358 mg), 67.3 \pm 2.6% (6730 \pm 255 mg), and 71.6 \pm 2.0% (7162 \pm 199 mg) of the total acetaminophen dose, respectively. At that point, no residues were present in the inner cylinder, so the experiment was not continued.

Soft Capsules

Similar to the hard capsules, the gelatin capsule shell started to rupture and release acetaminophen into the test medium within 2 minutes, forming a suspension.

Acetaminophen was released very rapidly when one capsule was tested: after 10 minutes, $96.9 \pm 0.6\%$ (485

 \pm 3 mg) of the dose had been released. In the FaSSGF buffer, 98.5 \pm 0.1% (492 \pm 0.5 mg) of acetaminophen was released, and no capsule residues were present in the inner cylinders.

When 10 capsules were tested, acetaminophen release was still very rapid. After 10 minutes in FaSSGF buffer, 86.1 \pm 0.9% (4304 \pm 47 mg) of acetaminophen was released. After 60 minutes, 90.6 \pm 0.7% (4529 \pm 34 mg) of the total dose had been released. Similar to the single dose test, no residual material was observed in the inner cylinder, so the experiment was not continued. The remaining 10% of the dose did not dissolve within 60 minutes of the experiment, even though the whole capsule content was exposed to the medium.

When 20 soft capsules were tested, dissolution was considerably slower than for one or 10 capsules. After 10 min, $46.9 \pm 7.8\%$ (4685 ± 778 mg) of the total dose was released. After 1 hr in FaSSGF buffer, $55.3 \pm 1.9\%$ (5533 ± 192 mg) of the drug had been released. Contrary to the lower doses, some residual material was present on the inner cylinder mesh, so the experiment continued in the FaSSIF-V2 buffer compartment where $72.5 \pm 12.8\%$ (7247 ± 1277 mg) of acetaminophen was released. No capsule residue was present in the inner cylinders, so the experiment was not continued. The remaining 27.5% of the total dose did not dissolve in FaSSIF-V2 buffer sections (100 min in total) despite the capsule content being completely exposed to the medium.

Similarity Factor f₂ Calculations

The f_2 results indicate that the profiles of one, 10, 20, and, for IR tablets, 50 dosage units of the same formulation differ from each other significantly. The only exception applies to the profiles of one vs. 10 soft capsules, where the f_2 value was 50.85, indicating that these two profiles differ from each other by less than 10% and can therefore be regarded as similar.

For all doses tested, the f_2 values of ER tablets compared to the IR tablets, hard capsules, and soft capsules are lower than 50, as might be expected when an ER formulation is compared with its IR counterparts. Among the IR formulations, f_2 results indicated similarity of the dissolution profiles when one dosage unit was tested. All other comparisons (except 20 IR tablets vs. 20 hard capsules) were well below the cut-off for similarity (f_2 = 50).

The f_2 analysis was supported by the ANOVA results, where the same doses were compared among the formulations.

Among one, 10, and 20 doses, the dissolution data showed statistically significant differences (p < 0.001) at the 10- and 60-min time points.

DISCUSSION

Solubility Testing

The aqueous solubility of acetaminophen was reported by Kalantzi et al. to be 14.7 mg/mL at 20 °C, 14.3 mg/ mL at 25 °C, and 23.7 mg/mL at 37 °C (*1*). Shaw et al. tested the solubility of acetaminophen in various buffers over the pH range 1.2–9 and reported values of 18.7 \pm 0.2–24.8 \pm 0.3 mg/mL (*11*). Our results (approximately 24 mg/mL at 37 °C) are in line with these data. Although polyvinylpyrrolidone (Povidone), which is listed among the excipients contained in the IR tablets, may positively influence the solubility of acetaminophen (*12*), it did not appear to influence the solubility of acetaminophen in the STADA tablets.

Dissolution Testing USP Apparatus 2

Preliminary experiments were performed in FaSSGF and FaSSIF-V1 buffers at a reduced volume (100 mL). The volume chosen is lower than the 250 mL specified for co-ingestion of water in bioequivalence studies, because patients often ingest drugs with just a few swallows of water. In the USP apparatus 2 setup, the experiments with a single IR tablet were performed under sink conditions, whereas dissolution from 10 IR tablets was limited by the solubility of acetaminophen. Similar limitations may also occur in vivo. Because dissolution in the FaSSGF and FaSSIF-V1 buffers was not complete in USP apparatus 2 at higher doses, the experiments were extended to better understand whether dissolution could be completed in more distal regions of the GI tract. To facilitate simulation of the changes in pH all the way along the GI tract, USP apparatus 3 was selected for further studies.

USP Apparatus 3

The rapid release of acetaminophen from three IR formulations (tablets, hard capsules, and soft capsules) when tested as a single dose is commensurate with the objective of providing rapid pain relief. Likewise, part of the dose in ER tablet is released rapidly to guarantee a rapid analgesic effect, which is then maintained for up to 8 hours by the ER part of the tablet to reduce the dosing frequency.

When higher doses were tested, acetaminophen was released more slowly and less extensively as the dose was increased. This tendency was consistent among all four acetaminophen formulations. In an extreme case, when 50 doses of the IR tablet were tested, the release slowed

2 Dissolution Technologies FEBRUARY 2024 www.dissolutiontech.com dramatically and only a small fraction (less than 10%) was released even when the tablets were subjected to conditions representative of passage along a major part of the GI tract. The failure of release can be traced back to extensive clumping of the tablets (Fig. 6).



Figure 6. Photograph of 50 IR tablets after 60 minutes in FaSSGF buffer in USP apparatus 3. Clumped tablet material filled nearly the entire volume of the inner cylinder. This was observed in all five compartments (up to 6 hours).

IR: immediate release; FaSSGF: fasted state simulated gastric fluid; USP: United States Pharmacopeia.

This observation might be linked to the clinically observed formation of a pharmacobezoar. Acetaminophen tablets have been reported to form a bezoar, e.g., in a case report of 70 tablets taken by a male patient (7 g acetaminophen in total, administered as a fixed dose combination with dihydrocodeine phosphate) (13). Pharmacobezoar formation was also demonstrated by Li et al. ex vivo (14). In stomachs removed from pigs, 75 or 100 IR tablets (37.5 or 50 g of acetaminophen) formed a bezoar when they were brought in contact with 28 mL of simulated gastric fluid in a water bath for 4 hrs, but 50 tablets (25 g) did not form the bezoar (14). Although pigs are often used as the model animal for in vivo drug absorption, it is important to note that there are some anatomical differences between pig and human stomachs, i.e. the pig stomach is two to three times bigger than the human stomach (15). In other work, Hoegberg et al. reported that 30 ER tablets formed a bezoar in contact with 1000 mL of simulated gastric fluid, but 30 IR tablets did not (16). The amounts of the four dosage forms, tested within our study, are lower than those studied in the ex vivo and in vitro reports, except for the test with 50 IR tablets. However, we used more physiologically relevant volumes in USP apparatus 3 than Hoegberg et al.

The rate and extent of release in our acetaminophen dissolution model is in line with clinical data. In the

late 1970s, Rawlins et al. studied acetaminophen pharmacokinetics after intravenous (IV) and oral administration in six volunteers (5). A dose of 1000 mg IV, as well as doses of 500, 1000, and 2000 mg orally, was administered in a crossover regimen with a 1-week washout period. The study showed incomplete bioavailability of acetaminophen after oral administration of these doses (all of which are under the recommended total daily dose) (5). The maximum plasma concentration after oral administration of 500 or 1000 mg acetaminophen was reached within 1 hour, but after oral administration of 2000 mg, the maximum plasma concentration was not reached until 2 hours, suggesting that the acetaminophen absorption was slower when a higher dose was administered.

Spyker et al. recently published a population pharmacokinetic (PK) model for acetaminophen based on data from randomized clinical trials of supratherapeutic IR doses and ER and modified release (MR) formulations as well as overdose case reports (17). The ER and MR formulations contained 650 mg acetaminophen; ER tablets had a 50:50 ratio of IR to ER whereas MR tablets had a 69:31 ratio, and the mechanism of release from the ER portion differed (17). Using the model, simulations of various acetaminophen doses (up to 100 g) for IR, ER, and MR formulations were performed. It was shown that increasing the acetaminophen dose would result in reduction of the absorption rate and bioavailability for all formulations. The model also suggested differences among formulations. After overdose, bioavailability values were lowest for MR formulations, greater for ER, and highest for IR (17). In the present study, data from the USP apparatus 3 method agree with the population PK model, with just 20% of the dose released from ER tablets within 6 hrs compared to 60% (hard capsules) or 100% (IR tablets) when 20 doses were tested. Thus, our in vitro model may be helpful in predicting clinical PK data after acetaminophen overdose, as only released drug can be absorbed. However, it is difficult to test intentional overdoses greater than 25 g acetaminophen in the in vitro model due to the size limitation of USP apparatus 3.

Most studies reported in the literature are patient cases and clinical trials involving IR or ER tablets. This study with USP apparatus 3 also included soft and hard capsules. These formulations had similar (hard) or faster (soft) dissolution rates than that of IR tablets. This might be important information regarding safety precautions for the marketed soft capsule formulations, because they are expected to be absorbed faster and more completely at higher doses than the tablets. Dissolution testing performed in USP apparatus 3 discriminated well between the four formulations. The ER tablet appears to be less prone to toxicity after overdosing. Importantly, the release and absorption of acetaminophen after intentional overdose (usually doses of 12–50 g) is shifted in time (2). Our results potentially link this shift to bezoar formation. So-called "double peak" (or "double hump") behavior has also been reported in the literature for acetaminophen pharmacokinetics in overdose cases. In the course of acetaminophen poisoning, a reduction of plasma concentration followed later by another rise in concentration was observed in some patients, regardless of the administration of an N-Acetyl cysteine antidote (*18, 19*). The second peak might possibly be caused by disruption of a bezoar.

CONCLUSION

A novel in vitro dissolution model was established for the release of acetaminophen when ingested in supratherapeutic quantities. The experiments were designed to reflect the physiology of the human GI tract in a fasted state and to simulate the ingestion of a single standard dose of acetaminophen as well as various degrees of overdose. Four acetaminophen products were compared: IR tablets, ER tablets, hard capsules, and soft capsules. The results obtained with the model are in line with published data obtained in acetaminophen clinical trials and showed important differences among the various formulations. Additionally, the in vitro model was able to model pharmacobezoar formation at very high doses, which has also been reported in the literature. This in vitro model for studying acetaminophen overdose may also be useful for testing other drugs, such as opioid analgesics.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article and may be requested by contacting the corresponding author.

DISCLOSURES

This work was funded by Fraunhofer Internal Programs (grant no. SME 840 046). The authors have no conflicts of interest to disclose.

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The Importance of Dissolution Tests to Evaluate Quality of Dietary Supplements: Case Study of Controlled-Release Caffeine Capsules

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ABSTRACT

Controlled-release capsules (named brand I and II) containing caffeine, available on the Brazilian and US market as dietary supplements, were assessed following the criteria described by the *United States Pharmacopeia*. The capsules were evaluated by average weight, caffeine content, disintegration, and dissolution tests. Test results for all capsules met the acceptance criteria for these tests, with the exception of the dissolution of brand I capsules. The release rate of an active pharmaceutical ingredient (API) from a dosage form, measured by a dissolution test, is one of the fundamental parameters leading to the formulation's feasibility. The dissolution test is not mandatory to approve a dietary supplement in Brazilian and US markets. This study highlights the importance of evaluating these products by means of a performance test, such as dissolution, using products containing caffeine as case study.

KEYWORDS: Caffeine, dietary supplements, controlled-release capsules, quality control, dissolution

INTRODUCTION

he market size of global dietary supplements was valued at \$151.9 billion US dollars in 2021 and is expected to expand at a compound annual growth rate (CAGR) of 8.9% from 2022 to 2030. The increasing consumer knowledge of personal health and wellbeing is expected to be a factor for dietary supplements growth over the projection period (1). Caffeine is one of the most consumed stimulants in the world and is a frequent ingredient in dietary supplements. This compound has several properties (Fig. 1): it is a central nervous system stimulant, a diuretic, it decreases fatigue, it enhances mental focus and athletic performance and presents thermogenic effects (2). There is also evidence proposing that the consumption of caffeine appears to reduce caloric intake, contributing to weight loss (3).

The safety dose for caffeine recommended by the United States Food and Drug Administration (FDA) and National Health Surveillance Agency of Brazil (ANVISA) is around 400 mg/day for adults from all caffeine sources, such as coffee, tea, pills, and others (*4*, *5*). High dosages (more than 400 mg/day) of this compound can cause severe hypertension, arrhythmias, seizures, and even death. Individuals who are more sensitive may present adverse effects at lower dosages. The complete absorption of caffeine occurs in the small intestine, and it needs around 45 minutes to reach 99% bioavailability, with no substantial first pass effect (5).



The human body eliminates caffeine within a few hours, leading some people to take caffeinated beverages or supplements recurrently over time. To retain the stimulating effects of caffeine and avoid an overdose, sustained-release systems have been developed and introduced on the market (6). Different formulations of caffeine-controlled release products are currently available for purchase in the US and Brazil.

The oral bioavailability of an active pharmaceutical ingredient (API) regularly depends on its dissolution upon ingestion, absorption in the small intestine, and transport to its target site of action (7). Dissolution is a precondition for absorption and in vivo efficiency for almost all compounds given in oral solid dosage forms. API absorption depends on its dissolution and solubilization under physiological conditions, and the permeability across the gastrointestinal (GI) tract. Because of the critical nature of the first steps, in vitro dissolution is an important and necessary tool to predict the human biological response (8). For this reason, dissolution testing is required for quality control of solid dosage forms containing APIs (medicines), but it is not mandatory for dietary supplements.

Dissolution testing is an important instrument for characterizing the performance of oral solid dosage forms. Its significance is founded on the point that for an API to be effective, it must first be released from the product and dissolve in the GI fluids previously absorption (\mathcal{B}).

The market of dietary supplements includes tablets, powders, and liquids. It is predicted that revenue from the tablet dosage form market will exceed \$100 billion US dollars (9). Capsules offer an alternative to tablets for oral delivery of therapeutic compounds. One advantage of capsules over tablets is flexibility to deliver not only solids but also non-aqueous liquids and semisolids as a unit dose solid dosage form. It can also be designed to delay the release of their contents into the GI tract, prolonging the therapeutic effect (10).

The aim of this work was to evaluate the quality of caffeine capsules available on the market as a modified-release formulation and to introduce the importance of performance tests such as dissolution for dietary supplements.

MATERIALS AND METHODS

Chemical Products and Capsules

The United States Pharmacopeia (USP) provided the reference standard used for caffeine. All solvents used were of analytical grade and purchased from Biotec

(Pinhais, PR, Brazil). Two different modified-release capsule brands were evaluated.

Brand I: Hard gelatin capsules containing caffeine microgranules (130 mg), water, tocopherol mix, safflower oil (500 mg), hydroxypropyl methylcellulose (HPMC), and silicon dioxide. According to the manufacturer, the delivery of caffeine occurs in two stages: 50% in the first hour and the remainder in the next 5 hours.

Brand II: Hard gelatin capsules containing caffeine beadlets (200 mg), sugar, gelatin, starch, food glaze, magnesium silicate, povidone, FD&C Yellow 6, and FD&C Red 40. According to the manufacturer, the release of caffeine occurs in a sustained manner for 8 hours.

The capsules are available in Brazil and the US. All analyses were conducted within the period of validity of the product.

Average Weight

The methodology described in the USP chapter <2091> Weight Variation of Dietary Supplements was used. Twenty intact capsules were weighed individually using an analytical balance (Shimadzu AW220, São Paulo, Brazil), and the average weight was further calculated. The results were compared with the USP requirements (*11*).

Caffeine Content

Ten capsules of each brand were opened and their contents (microgranules for brand I or beadlets for brand II) were removed, weighed, and crushed. An amount of the content equivalent to 50 mg of caffeine was weighed, transferred to 100-mL volumetric flask and the volume completed with distilled water. These flasks were shaken for 20 minutes in an ultrasonic bath and diluted as necessary. Caffeine content was determined by UV spectrophotometry at 237 nm using a UV spectrophotometer (Varian Cary 50). The UV spectrophotometric quantification method was adapted from Tan and colleagues and validated by linearity, precision, quantification and detection limits, and specificity (*6*).

The linear equation was achieved using weighed and diluted caffeine to obtain solutions in the range of $0.8-25.0 \ \mu g/mL$. After analysis in a UV spectrophotometer at 237 nm, a chart provided the linear equation: $y = 0.0523 \ x - 0.006$ and r = 1, limit of quantification: $0.29 \ \mu g/mL$, and limit of detection: $0.10 \ \mu g/mL$.

Disintegration Tests

Capsule disintegration tests were carried out in a USP disintegration apparatus (Nova Ética, 301-AC, São

Paulo, Brazil) in distilled water at 37 °C. The time for disintegration of each unit (n = 6) was recorded (11).

Dissolution Tests

Dissolution studies were performed using a Varian VK 7000 dissolution tester, equipped with USP apparatus 1 (basket). Samples (n = 6) were analyzed in two different dissolution media: a) 900 mL of distilled water and b) 900 mL of 0.1 N hydrochloric (HCl) acid. Dissolution studies were conducted at 37 °C and stirring speed of 50 rpm. Samples were collected at defined time intervals for 480 min, filtered with a 45-µm PVDF filter (Filtrilo), diluted to fit the equation curve, and quantified by UV spectrophotometry.

The dissolution efficiency was calculated by software DD Solver. One-way analysis of variance (ANOVA) and Tukey's multiple comparisons test were employed to test the statistical significance regarding the dissolution efficiency of samples. Differences were considered significant for p < 0.05 with a confidence level of 95%. The results were analyzed using Excel.

Dissolution Kinetics

The results obtained from the dissolution tests were used to evaluate the dissolution kinetics of caffeine from the capsules. The straight-line equation and linear regression were used to determine the percentage of dissolved caffeine as a function of time. The kinetics models applied are described in Table 1, and the best model was selected based on coefficient of correlation analysis (R^2) of linear regression (12–14).

Table 1. Dissolution Kinetic Models Applied to Caffeine Capsules								
Model	Mathematical Equation	Release Mechanism						
Zero order	$C = C_0 - K_0 t$	Diffusion mechanism						
First order	Log C= log C ₀ -K ₁ t/2.203	Fick´s first law, diffusion mechanism						
Higuchi	Q ₀ /Qt=K _{H.} t ^{1/2}	Diffusion medium-based mechanism in Fick´s first law						
Korsmeyer-Peppas	$Ct/C_{\infty}=K_{k.}t^{n}$	Semi-empirical model, diffusion-based						

RESULTS AND DISCUSSION

Average Weight, Caffeine Content, and Disintegration Time

The weight determination indicates if the units of a batch show weight homogeneity. The average weight of the capsules was 832 ± 5.5 mg and 520 ± 2.0 mg for brands I and II, respectively. Caffeine content was $99.7 \pm 4.1\%$ and $98.9 \pm 3.2\%$ for brands I and II, respectively. According to the USP, the requirements of average weight are met if each of the individual weights is within the limits of 90– 110% of the average weight. Capsules from both brands had variations in their weights within the specified limits, as well as presented a caffeine content in accordance with the amount stated on the label.

The time for disintegration of capsules was 5 minutes for both samples, indicating the rapid liberation of their content into the aqueous medium. Disintegration and dissolution tests are described in *USP* general chapter <2040> as a quality control tool to routinely assess the performance of dietary supplements, which states that hard-shell capsules must be completely disintegrated within 30 minutes (*11*). As both samples showed a shorter disintegration time, the caffeine release could be governed by the formulation of granules/beadlets and not by the capsule itself.

Dissolution Test

The dissolution test is a performance assay applied to different pharmaceutical formulations to evaluate their drug release from the pharmaceutical form (15). Based on release profiles shown in Figure 2, brand I released less than 5% of the caffeine content in 8 hours in both media evaluated. Brand II showed a continued caffeine release over time, dissolving around 90% of caffeine content in 8 hours, in both water and acidic media.



The evaluation of dissolution is mandatory for medicines but is not required to register a product in the FDA or ANVISA as a dietary supplement. As there are no acceptance criteria to assess the performance of these products, we considered the release claimed by the brands on their labels. Brands I and II claimed to release the caffeine content over 5 and 8 hours, respectively. In controlled-release systems, the drug is released or activated at predetermined intervals or gradually released over a period of time, as was observed for brand II release profiles (Fig. 2) (*16*). However, the low caffeine release of brand I indicates that the composition of the capsules interferes with the release mechanism.

Caffeine is a weak acid with pKa of 14.0 and lipophilicity (octanol-water partition coefficient, LogP) of 0.1 (17). Its water solubility is 11.0 mg/mL, being classified as class I (high solubility and high permeability) according to the Biopharmaceutical Classification System (18). The dosages of capsules used in the tests were 130 and 200 mg for brand I and II, respectively, and the volume of both media was 900 mL. This means that the sink condition (volume of solvent 5–10 times greater than the volume present in the saturated solution) was kept in the bulk solution during the test, so the low caffeine release from brand I was not due to saturation effects outside the pharmaceutical dosage.

Brand I was composed of different oils that covered the HPMC granules containing caffeine. The release of caffeine from the capsule was probably affected by the surrounding barrier formed by the oil, preventing the dissolution media from accessing the granules and the hydrophilic caffeine passing through the oil layer (Fig. 3). Controlled drug release from a hydrophilic matrix based on HPMC follows several types of physical phenomena, such as water, drug, and polymer chain diffusion, polymer swelling, and subsequent dissolution of drug and polymer. Even in the case of freely water-soluble molecules, such as diprophylline and theophiline, saturation solubility effects can occur within the dosage form (while providing sink conditions outside), impeding drug release (*19*).



Figure 3. Images of capsules named Brand I and Brand II on left and right, respectively.

Dissolution Kinetics

The quantitative interpretation of the values obtained from the dissolution tests was simplified by using mathematical models to describe the drug release from the pharmaceutical form (Table 2). Because brand I did not release at minimum 60% of caffeine, it was not possible to calculate the best model to describe the caffeine release (Table 2). For brand II, the best fit model was the Korsmeyer–Peppas equation. In this equation, Mt/M[∞] characterizes the fraction of permeated drug, t is time, K is the transport constant (dimension of time⁻¹), and n is the transport exponent (dimensionless) (13, 20). The values of n calculated for brand II in water and HCl 0.1 N were 1.14 and 1.22, respectively. Values of n > 1 are related to super case II kinetics, wherein multiple mechanisms are involved in drug release, such as diffusion, swelling, relaxation, and erosion (13, 20).

In addition, dissolution efficiency (DE) was employed to compare the dissolution profiles of the brand II capsules in different media (*21*). The time *T* in this study was 480 minutes (8 h). The DE values for brand II were similar in both media (Table 2).

The dissolution of an active ingredient administered in the solid state is a prerequisite for efficient and subsequent transport within the human body, which underscores the importance of dissolution tests for dietary supplements. Considering the high complexity of a component release from a controlled-release system, even the release of freely water-soluble molecules should not be taken for granted. In the case of caffeine, ineffective release of the active ingredient from the capsules during the time shown on the label could induce the patient to take more, contributing to possible toxicity and even lethality (i.e., most commonly via myocardial infarction or arrhythmia) if enough caffeine is consumed (*22*).

Gusev and colleagues evaluated the applicability of *USP* <2040> protocols for disintegration and dissolution testing of dietary supplements containing green tea available in the US market (*23*). The results indicated that in dissolution testing, for the release of epigallocatechin-3-gallate (EGCG), the most abundant of the green tea catechins, only 6 out of 20 dietary supplements were approved. These results raise concerns that EGCG was

Table 2. Dissolution Efficiency and Mathematical Model Parameters (R²) for Controlled-Release Caffeine Capsules

	DE (%)	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer-Peppas
Brand II - Water	43.78 ± 2.24*	0.9944	0.9581	0.9287	0.9819	0.9970
Brand II - HCl 0.1 N	45.68 ± 3.18*	0.9802	0.9686	0.9225	0.9797	0.9892

*Statistically similar (p < 0.05) in different media for the same brand.

not released properly from green tea dosage forms of dietary supplements (23).

As the dietary supplement industry grows, the risk of interactions between prescription medications and dietary supplements may increase. In the US, approximately 80% of adults over 50 years take at least one prescription medicine, and more than 20% take at least five prescription medications, and more than half of these patients also use dietary supplements (9).

The brief case study of controlled-release capsules containing caffeine presented herein demonstrates a need to look at dietary supplements (in capsule or tablet form) with the same quality and safety criteria that the regulatory agencies use when assessing a medicine.

CONCLUSIONS

Regulatory agencies such as ANVISA and the US FDA do not require the dissolution test for dietary supplements. Dissolution studies of two brands of controlled-release capsules containing caffeine indicated that one brand did not match the specification described on the label of the product. Differences in caffeine dissolution can lead to serious health problems from undesired intoxication or overdose, owing to absence of the desired effect. This case study raises an alert and supports the need to perform dissolution tests on products sold as dietary supplements in the form of tablets and capsules.

DISCLOSURES

The authors received no financial support for this work and have no conflicts of interest to disclose.

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Evaluation of a pH-Gradient Biphasic Dissolution Test for Predicting In Vivo Performance of Weakly Basic Drugs

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ABSTRACT

The present study aimed to confirm the biorelevance of the pH-gradient biphasic dissolution model for three ketoconazole (KTZ) formulations with different excipients and establish an in vivo-in vitro correlation (IVIVC). Experiments were performed with a pH-gradient biphasic dissolution model for drug absorption, consisting of a sequential pH-gradient in the aqueous phase and octanol phase, representing the stomach, duodenum, jejunum and ileum compartments, and small intestinal membrane, respectively. Conventional single phase in vitro dissolution tests with and without pH-shift lacked discrimination. The pH-gradient biphasic dissolution test showed discriminatory power for the three KTZ formulations, with the same ranking of drug release in vitro and in vivo. A good IVIVC was established between in vitro release data in octanol and in vivo data in rats, demonstrating the in vivo biorelevance of the pH-gradient biphasic dissolution model. This study presents a promising approach for predicting in vivo performance of weak bases containing formulations in early drug development.

KEYWORDS: pH-gradient biphasic dissolution test, in vitro-in vivo correlation, weakly basic drugs, biorelevance, oral bioavailability, dissolution

INTRODUCTION

ral absorption of weakly basic drugs is a dynamic complex process mainly influenced by physicochemical properties of the drug and physiological conditions in the gastrointestinal (GI) tract, especially for dynamic pH conditions. The pH values in the fasted stomach usually in the range of 1.5–2.0, with a transit time of 0.5-2 h (1, 2). Average pH values in the fasted upper small intestine are 5.0-7.5, including pH 5.0-6.5 in the duodenum, pH 6.0-7.0 in the jejunum, and pH 6.5-7.5 in the ileum (3, 4). Transit time in the small intestine is often considered to be approximately 4 h (5). Weakly basic drugs can quickly dissolve at gastric pH, but not at intestinal pH following a supersaturationprecipitation process in the small intestine, which shows limited absorption. Conventional in vitro dissolution tests lack biorelevancy with in vivo dissolution (6, 7). Many attempts have been made to overcome the limitations and better predict bioperformance of oral products by creating different biorelevant dissolution methods (8–11). The biphasic dissolution test has exhibited improved in vivo prediction by incorporating an absorptive sink. In this technique, the drug first dissolves in the aqueous phase to simulate dissolution at gastric pH, then the dissolved drug immediately partitions in the organic phase to mimic drug absorption through the intestinal membrane. This biphasic dissolution model enables the evaluation of various formulation factors such as particle size, drug loading, wettability, polymorphic forms, and drug precipitation (*12–19*).

In our previous report, a pH-gradient biphasic dissolution system was developed through an orthogonal test design with three factors and three strengths of ketoconazole (KTZ) to simulate pH conditions in the stomach, duodenum, and jejunum and ileum in the aqueous phase and to mimic an intestinal absorptive sink in the octanol phase (20). The aim of the current study was to confirm the biorelevance of the pH-gradient biphasic dissolution model for three KTZ formulations with different excipients compared to conventional dissolution tests. Subsequently, an animal study with rats was also performed to evaluate the relationship of the in vitro and in vitro dissolution profiles.

METHODS

KTZ was purchased from Wuhan Dahua Weiye Medicine Chemical Co., Ltd. (Wuhan, China). Lactose monohydrate was donated by PinTech Pharmaceutical Co., Ltd. (Shanghai, China). β -cyclodextrin (β -CD), microcrystalline cellulose (MCC), 1-octanol, hydrochloric acid (HCl), sodium dihydrogen phosphate dihydrate, sodium hydroxide (NaOH), tribasic sodium phosphate, and sodium chloride were obtained from Sichuan Kelun Pharmaceutical Co., Ltd. (Chengdu, China). Hard gelatin capsules (size 0) were donated by Suzhou Capsugel Ltd. (Suzhou, China). All other reagents used were of analytical grade.

Preparation of KTZ Formulations

Three formulations were prepared by mixing 100-mg KTZ with lactose, β -CD, and MCC at a weight ratio of 1:1. Powder blends were filled in size 0 hard gelatin capsules.

Conventional USP Single-pH Dissolution Test

The compendial dissolution test was performed in 900 mL 0.1 N HCl at 50 rpm and 37 °C in a USP apparatus 2 (paddle) (RCZ-8, Shanghai Huanghai Drug Inspection Instrument Co., Ltd, Shanghai, China) (n = 3). Samples were collected at predetermined time intervals and measured using a UV-spectrophotometer (T6, Beijing Puxi General Instrument Co. Ltd, Beijing, China) at 224 nm.

Conventional USP pH-Shift Dissolution Test

To evaluate the effect of pH change, drug release was assessed according to the *USP* general chapter <711> enteric dissolution test (method A) in USP apparatus 2. The KTZ formulations were first tested in 750 mL of 0.1 N HCl for 2 h followed by a pH adjustment to 6.8 ± 0.05 by adding 250 mL of 0.2 M tribasic sodium phosphate (n = 3). Samples were withdrawn at predetermined time points and used for UV spectrophotometry.

pH-gradient Biphasic Dissolution Test

Based on our previous study, the developed pH-gradient biphasic dissolution test (Fig. 1) was used to assess three KTZ formulations (20). Briefly, each formulation containing 100 mg KTZ with a sinker was added into 250 mL of gastric buffer (pH 2.0) for 30 min, then the aqueous medium was adjusted to pH 5.5 to mimic the duodenum by adding 5 M NaOH and 100 mL of presaturated 1-octonal as the upper organic phase to simulate the intestinal membrane (n = 3). Subsequently, the aqueous phase was readjusted to pH 6.5 to mimic the jejunum for 2 h, then the final pH was increased to 6.8 for 1 h. The rotating speed was set to 30 rpm. The temperature was maintained at 37 °C. Samples were withdrawn manually from both the aqueous and organic phases at predetermined time points and replaced with the same volume of fresh media. The aqueous samples were passed through a 0.45- μ m Durapore membrane filter, and the organic samples were centrifuged at 12,000 rpm for 20 min (TG-16, Gongyi Yuhua Instrument Co. Ltd, Gongyi, China). Drug concentrations in aqueous and organic phases were determined by UV spectrometry at 224 nm.



In Vivo Studies

Animal studies were approved by the local ethical committee at the Third Military Medical University, Chongqing, and performed in accordance with guidelines of experimental animal care. Female Sprague-Dawley rats weighing 200–250 g were fasted for 12 h before drug administration. Each KTZ formulation was dispersed in deionized water prior to dosing and administered by oral gavage at a dose of 45 mg/kg (n = 5). Blood samples were collected from retro orbital choroid plexus under mild anesthesia at 0, 1, 2, 3, 4, 6, 8, 12, and 24 h after dosing and placed into heparin pretreated tubes. The blood samples were centrifuged at 3500 rpm for 10 min, and plasma was stored at –20 °C until further analysis.

Plasma concentration of KTZ was determined by highperformance liquid chromatography (HPLC) analysis. Samples were analyzed using the Agilent HPLC system (1260 Infinity, Agilent, Germany) equipped with an Ultimate XB-C18 column (250 × 4.6 mm, 5 μ m, 120 Å) maintained at 25 °C. The mobile phase was a mixture of acetonitrile and 0.02 M phosphate buffer at pH 6.8 (65:35, v/v) at the flow rate of 1.0 mL/min, and the UV detector was set to 254 nm (*21, 22*).

Pharmacokinetic Analysis

The pharmacokinetic (PK) parameters, including area under the plasma concentration time curve from 0 to 24 h (AUC_{0-24 h}), the time to reach maximum plasma concentration (T_{max}), and the peak plasma concentration of drug (C_{max}) after administration of KTZ formulations in rats were determined using a non-compartmental model analysis by a freely available add-in program for Microsoft Excel, PK Solver (23).

Statistical Analysis

All data were expressed as mean \pm standard deviation (SD). The results were compared by one-way analysis of variance (ANOVA), and p < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Conventional USP Single-pH Dissolution Test

As shown in Figure 2, all three KTZ formulations had similar dissolution profiles in the single-pH dissolution test, and they dissolved more than 80% at 10 min. The compendial dissolution test lacked discrimination between these KTZ formulations due to fast drug dissolution.



Figure 2. Dissolution profiles (mean \pm SD; n = 3) of three ketoconazole (KTZ) formulations under sink conditions (900 mL 0.1 N HCl): (\bigcirc) KTZ-lactose, (\blacksquare) KTZ-8-CD, and (\blacktriangle) KTZ-MCC.

Conventional USP pH-Shift Dissolution Test

KTZ is classified as a weakly basic Bipharmaceutical Classification System (BCS) class II drug with a diphasic pK_a (2.94 and 6.51) and a log P value of 3.73, so drug solubility in a pH-dependent manner is reported as 20.3 mg/mL in simulated gastric fluid (pH 1.2) and 6 μ g/mL in simulated intestinal fluid (pH 6.8), respectively (24). Thereby, a pH-shift dissolution test was used to assess the influence of pH change throughout the GI tract. All three KTZ

34 Dissolution Technologies FEBRUARY 2024 www.dissolutiontech.com formulations showed similar dissolution profiles in 0.1 N HCl for the first 2 h. However, drug concentration of KTZ decreased after the pH change, which was attributed to fast precipitation due to much lower solubility at neutral pH (Fig. 3). Unexpectedly, drug concentration at pH 6.8 was almost constant over time. The dissolved excipents in the phosphate buffer would facilitate dissolution of precipitated KTZ, leading to a concentration plateau, or the interactions between KTZ and lactose, β -CD, or MCC might occur via hydrogen bonding to delay crystallization (*25, 26*).



Figure 3. Dissolution profiles (mean \pm SD; n = 3) of three ketoconazole (KTZ) formulations under pH-gradient conditions (750 mL 0.1 N HCl for 2 h followed by a pH adjustment to 6.8 \pm 0.05 with addition of 250 mL 0.2 M tribasic sodium phosphate solution) (dashed vertical line represents the pH change): (\bullet) KTZ-lactose, (\blacksquare) KTZ- θ -CD, and (\blacktriangle) KTZ-MCC.

pH-Gradient Biphasic Dissolution Test

Each formulation (containing 100 mg drug) maintained sink conditions (< 20% of drug solubility ($C_s = 5.6 \text{ mg/mL}$) in 100 mL octanol (20). All KTZ formulations dissolved fast and reached 100% release in the gastric buffer at pH 2.0 (Fig. 4A). After pH change, drug concentrations significantly decreased to a plateau in the aqueous phase due to immediate precipitation and partitioning into the organic phase of drug. In contrast, the corresponding dissolution profiles in the organic phase differed, with a ranking of KTZ-lactose > KTZ- β -CD > KTZ-MCC (Fig. 4B). Thus, these KTZ formulations were well-discriminated in the organic phase of the pH-gradient biphasic dissolution test. KTZ-lactose showed the highest dissolution profile in the organic phase, which could be the result of maintaining the most free drug in the aqueous phase and quickly partitioning into the organic phase. The hydrogen bonds forming between KTZ and lactose could retard recrystallization, or the formation of smaller dispersed drug particles redissolve by de-agglomeration due to the hydrophilicity of fine lactose (25, 27). Although β -CD had good solubilization for KTZ and displayed a slightly higher drug concentration in the aqueous phase, drug concentration in the organic phase was lower compared to KTZ-lactose. This was because solubilized drugs that form cyclodextrin complexation may have limited permeability due to the decreased free fraction of the drug available for membrane permeation (28, 29).

Other studies have reported inconsistent results between in vitro dissolution and in vivo absorption (*30, 31*). Compared with lactose and β -CD, MCC as a hydrophobic carrier would be expected to perform inferiorly (*32*). Another reason could be immobilizing of KTZ molecules on the MCC surface by hydrogen bonding and facilitating heterogeneous nucleation owing to MCC having a heterosurface (*33*). Given the continuous concentration gradient between two phases, the differences of dissolution and precipitation kinetics in the aqueous phase could be magnified by the presence of an organic phase (*15*).

In Vivo Study

To evaluate in vivo PK performance of the three KTZ formulations, a non-crossover study in rats was conducted. The PK parameters are summarized in Table 1. Significant differences were found between the C_{max} and AUC_{0-24h} values of the three KTZ formulations (p < 0.05). The same rank order of drug release observed in the pH-gradient biphasic dissolution test (KTZ-lactose > KTZ- β -CD > KTZ-MCC) was consistent with the C_{max} and AUC values of KTZ-MCC, KTZ- β -CD, and KTZ-lactose.

 Table 1. Pharmacokinetic Parameters for Three Ketoconazole (KTZ)
 Formulations in Rats After Oral Administration (45 mg/kg)

Formulation	C _{max} (µg/mL)	AUC _{0-24h} (μg h/mL)	T _{max} (h)			
KTZ-MCC	1.52 ± 0.22ª	12.61 ± 1.78ª	2.60 ± 0.89			
KTZ-β-CD	$3.19 \pm 0.46^{a,b}$	19.62 ± 3.11 ^{a,b}	1.80 ± 0.34			
KTZ-lactose 3.50 ± 0.41 ^b 27.97 ± 4.41 ^b 2.80 ± 0.45						
Values are expressed as mean ± SD, n = 5.						

^ap < 0.05 vs. KTZ-lactose; ^bp < 0.05 vs. KTZ-MCC

In Vitro-in Vivo Correlation

A level C in vitro-in vivo correlation (IVIVC) was tested using the percentage of KTZ dissolved in both aqueous and organic phases in the biphasic test at 3 h vs. an in vivo parameter (AUC or C_{max}). No meaningful IVIVC was obtained in the aqueous phase at 3 h and AUC_{0-24h} or C_{max} (Figs. 5A and 5B); however, good linear relationships were obtained in the organic phase at 3 h and the in vivo C_{max} ($R^2 = 0.96$) and AUC_{0-24h} ($R^2 = 0.92$) (Figs. 5C and 5D). This pH-gradient biphasic dissolution system thus reflected both in vitro and in vivo dissolution kinetics of the three KTZ formulations with different excipients, and the release profiles from the organic phase could serve as an indicator for in vivo drug performance.

CONCLUSIONS

Compendial dissolution tests lacked discrimination and in vivo prediction for three KTZ formulations, including the conventional pH-shift dissolution test. Conversely, the pH-gradient biphasic dissolution system showed discriminatory power for the KTZ formulations with different excipients. A good IVIVC was obtained between in vitro dissolution in the organic phase and in vivo



Figure 4. Dissolution profiles (mean \pm SD; n = 3) of three ketoconazole (KTZ) formulations determined from the (A) aqueous and (B) organic phases in the pH-gradient biphasic dissolution test (gastric buffer pH 2.0 for 30 min followed by a pH adjustment to 5.5 \pm 0.05 for 30 min, 6.5 \pm 0.05 for 2 h, and 6.8 \pm 0.05 for 1 h, respectively) (dashed vertical lines represent the pH changes): (\bullet) KTZ-lactose, (\blacksquare) KTZ-6-CD, and (\blacktriangle) KTZ-MCC.



Figure 5. Relationships of in vitro dissolution (mean \pm SD) of three ketoconazole (KTZ) formulations with different excipients in the aqueous and organic phases of the biphasic test at 3 h plotted against in vivo C_{max} (A and C), or AUC (B and D) values: (•) KTZ-lactose, (•) KTZ- β -CD, and (•) KTZ-MCC (n = 3 for in vitro studies; n = 5 for in vivo rat studies).

performance in rats. The pH-gradient biphasic model has great potential for weakly basic BCS class II drugs in the early development of formulations.

ACKNOWLEDGEMENT

The authors would like to thank the Third Military Medical University, Chongqing, for the support with the animal studies.

DISCLOSURES

This work was financially supported by the National Natural Science Foundation of China (grant no. 82202340), Science and Technology Research Program of Chongqing Municipal Education Commission (grant no. KJQN202100833), Chongqing Research Program of Basic Research and Frontier Technology (grant no. CSTB2022NSCQ-MSX1420), High-level Talent Research Start-up Project of Chongqing Technology and Business University (grant no. 1956039), Scientific Research Project of Chongqing Technology of Natural Nedicine Research of Chongqing Education Commission (grant no. KFJJ2019093), and Open Project of Central Nervous System Drug Key Laboratory of Sichuan Province

(grant no. 200024-01SZ).

The authors have no conflicts of interest to disclose.

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Evaluation of Different Methods for Dissolution Profile Similarity Comparison of Montelukast Tablets in Türkiye

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ABSTRACT

This study aimed to evaluate the highly variable in vitro dissolution profiles of generics and innovator montelukast products in the Turkish drug market by comparing model-dependent and model-independent analysis methods. Seven generic montelukast sodium products were tested, labeled G1–G7, and compared with the innovator. Dissolution tests were carried out with United States Pharmacopeia (USP) apparatus 2 (paddle) in 900 mL of distilled water containing 0.5% sodium dodecyl sulfate (SDS), fasted state simulated intestinal fluid (FaSSIF), or fed state simulated intestinal fluid (FeSSIF). The most accepted and used model for dissolution profile comparison of regulations in the world is the model-independent similarity factor (f_2); however, in this study, the bootstrap f_2 confidence interval method was also used due to highly variable dissolution data. The values of f_2 , $\hat{f}_{2,exp}$, and $\hat{f}_{2,bc}$ were calculated with DDSolver, Bootf2BCA, and PhEq_bootstrap software. DDSolver was also used for model-dependent calculations. Two generic products showed similarity with the innovator ($f_2 > 50$) in biorelevant media (G4 in FeSSIF and G7 in FaSSIF); however, satisfactory results were not obtained in 0.5% SDS. The observed differences may be due to the dissolution method or nature of montelukast sodium (i.e., pH-dependent, poorly water-soluble, first-pass metabolism-exposed drug).

KEYWORDS: montelukast, DDSolver, Bootf2BCA, PhEq_bootstrap, f_2 bootstrap methods, dissolution

INTRODUCTION

sthma is a chronic inflammatory respiratory disease characterized by inflammation of the airways, which causes swelling and narrowing of the airways. The disease pathogenesis mostly involves interactions between inflammatory mediators such as cytokines, cysteinyl leukotrienes, and environmental factors (1). Montelukast is a selective leukotriene receptor agonist that inhibits the cysteinyl leukotriene receptor 1, and it has been approved by many national authorities, including the United States Food and Drug Administration (FDA) and the Turkish Medicines and Medical Devices Agency (TITCK), for treatment and prophylaxis of diseases such as seasonal allergies and bronchospasm (2).

Montelukast is an acidic and lipophilic substance with solubility in water of 0.2–0.5 μ g/mL at 25 °C (*3*). Montelukast is classified as a class IIa compound according to the Biopharmaceutics Classification System (BCS) due to low solubility, high permeability, and weak acid structure (*4*). Because of the low water solubility

of montelukast, the salt form, montelukast sodium (MS), is generally used. MS undergoes hepatic first-pass metabolism (3, 5). Commercially available dosage forms of MS include film-coated tablets, chewable tablets, and powders containing granules. For solid dosage forms, dissolution is important in optimizing the drug manufacturing process, maintaining the same quality in production across all batches, evaluating pre- and post-approval changes, predicting in vivo drug behavior, and determining bioequivalence and therapeutic equivalence between innovator and generic products. The most used approach to examine the effect of dissolution studies under conditions determined by various guidelines and pharmacopeia (6, 7).

The dissolution test and dissolution profile comparison are important tools for drug development and regulatory approval. The most common method used to compare dissolution profiles is the similarity factor (f_2). Because it is easy to use and calculate, it has been accepted by many regulatory authorities in the world in a short time for comparison of dissolution profiles (7–9). Although it is a simple method, some conditions must be met for the use of f_2 . The profile should contain at least three dissolution time points other than 0; 12 units should be tested for each innovator and generic; the total cumulative percentage of dissolved drug should be above 85%; and the coefficient of variation for the dissolution points being compared should be less than 20% at the first time point and less than 10% at the other time points (6). Disadvantages of f_2 include unknown sample distribution, not reflecting the location of change, being easily affected by a change in the number of time points, and not considering high variability (7).

In recent years, different methods have been evaluated for comparison of variable dissolution profiles. Among these, a 90% confidence interval (CI) of f_2 has been proposed as a possible approach for profile comparison based on bootstrap methodology, where f_2 is not a point estimator, to assesses the similarity of dissolution profiles with high variation (10, 11). The f_2 bootstrap method is generally preferred by the US FDA and European Medicines Agency (EMA) (6, 7, 10).

This study aimed to compare dissolution profiles with high variability at early time points for innovator and generic MS tablets available in the Turkish drug market using model-independent (f_2 similarity and f_2 bootstrap methods) and model-dependent approaches.

MATERIALS AND METHODS

Materials

MS was provided by Enaltec (India). Innovator (Singulair 10 mg film-coated tablet) and generic MS tablets were bought from different pharmacies in the Turkish drug market. The chemicals and reagents used to perform the experiments included sodium dodecyl sulfate (SDS, Tekkim, Türkiye), sodium hydroxide pellets (NaOH, Sigma Aldrich, USA), monobasic sodium phosphate monohydrate (NaH₂PO₄·H₂O, Merck, Germany), sodium chloride (NaCl, Merck, Denmark), hydrochloric acid (HCl, Isolab, Germany), glacial acetic acid (CH₃COOH, Sigma Aldrich, USA), and simulated intestinal fluids (SIF) powder (Biorelevant, UK).

In Vitro Dissolution Studies

In vitro dissolution studies were carried out using United States Pharmacopeia (USP) apparatus 2 (paddle) (Sotax Unit-AT 7 Smart, Switzerland) at 50 rpm and 37 \pm 0.5 °C. The dissolution apparatus was wrapped with aluminum foil during the studies to protect MS from light. The dissolution studies were conducted using

900 mL distilled water containing 0.5% SDS, which is the FDA-recommended dissolution media, fasted state SIF (FaSSIF), or fed state SIF (FeSSIF) because the FDA recommended that bioequivalence studies of filmcoated tablets containing montelukast be performed under fasting or fed conditions (12). FaSSIF and FeSSIF are biorelevant media that contain different amounts of sodium taurocholate and phospholipids to simulate the in vivo fasted and fed states. These media were prepared according to the protocols of Biorelevant.com. Samples were withdrawn at predetermined times (5, 10, 20, and 30 min). An equal volume of fresh medium was added to maintain sink conditions. The samples were filtered using a 0.45-µm membrane filter, and the concentration of MS in samples was determined by UV spectrophotometry (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Finland) at 359 nm. The dissolution profiles were evaluated by the cumulative percentage of drug dissolved over time, reported as mean \pm standard deviation (SD) (n = 3).

Data Analysis

Three software programs were used to evaluate similarity of the dissolution profiles: DDSolver for model-dependent evaluation (f_2); DDSolver, Bootf2BCA_v1.3, and PhEq_ bootstrap v 1.2 for model-independent evaluation (f_1 [difference factor], f_2 , and f_2 bootstrap).

DDSolver is an easy-to-use Microsoft Excel add-in program that is often used for the comparison of dissolution profiles.

Bootf2BCA_v 1.3 is an open-source software developed with the R statistics environment. Statistical analysis and graphical evaluation were performed using R (V 4.1.3) and RStudio (V 2022.02.1). This program includes four different types of confidence intervals when determining the expected parameters (i.e., the normal approximation interval, base bootstrap interval, percentile interval, and bias-corrected and accelerated [BCa] interval). The program includes advanced parameters such as dissolved amount (Q) \geq 85% auto cut-off rule options, sampling mode (individual values, whole profiles), boot package simulation type (ordinary and balanced), statistic (selection of statistic to be bootstrapped), and seed (setting the value of seed for pseudorandom numbers).

PhEq_bootstrap v 1.2 was developed in the Lazarus environment, it is a program coded in Pascal (13). The program consists of three parts (main, graph, and about). It has two options for sampling (individual values and whole profiles), and each dissolution profile has options for a default rule of Q above 85% and bootstrap of 5000.

PhEq_bootstrap calculates f_2 , expected f_2 ($\hat{f}_{2,exp}$), and bias-corrected f_2 ($\hat{f}_{2,bc}$), and gives a 90% CI for $\hat{f}_{2,exp}$, although the type of CI is not explicitly specified.

Suitability of the dissolution profiles to fit kinetic models was determined by the adjusted coefficient of determination (R^2_{adj}), Akaike information criterion (AIC), and model selection criterion (MSC). The model with the highest R^2_{adj} and MSC and the lowest AIC were determined as the most suitable model (14). In addition, different f_2 estimators and various bootstrap CIs (calculated based on 5000 bootstraps) were evaluated.

Tablet Characterization Studies for Quality Control

Within the scope of quality control, the appearance, weight variation, content uniformity, hardness, and disintegration time were analyzed according to USP guidelines.

Validation Studies

The validation and analytical studies were performed according to the USP and ICH Q2 guidelines (*15*). Results were within acceptable limits for all parameters.

RESULTS

In Vitro Dissolution Profiles

Dissolution studies are used to predict the in vivo behavior and therapeutic efficacy of active substances such as MS prior to conducting in vivo bioequivalence studies. MS is a weakly acidic active substance with pH-dependent solubility. Although MS has low solubility at pH 1.2-4.5, its solubility increases as pH increases, there is no significant difference in solubility between pH 5 and 7.5 (3, 4). This situation is similar to the results of the current study. Namely, no pH-dependent increase in dissolution was observed in 0.5% SDS, FaSSIF, or FeSSIF media with pH values of 7, 6.5, and 5, respectively. More than 85% of MS was dissolved within 30 minutes in 0.5% SDS for all products except G1; however, this differed for biorelevant media. More than 85% of MS dissolved within 30 minutes in FaSSIF for the innovator, G1, G6, and G7 tablets and in FeSSIF for the innovator, G1, G3, G4, G5, and G7 tablets (Figure 1). High variability was observed at the early time points (i.e., coefficient of variation was > 20 for the first time points and > 10 for subsequent time points).

Results of the profiles evaluated with DDSolver, DDSolver bootstrap, Bootf2BCA and PhEq_bootstrap are presented in Tables 1–3 (data for DDSolver evaluation are not shown). When all results were compared, f_2 , and f_2 bootstrap values calculated using DDSolver, Bootf2BCA, and PhEq_bootstrap differed in all dissolution media. For MS release in 0.5% SDS, similarity with the innovator ($f_2 > 50$) was demonstrated by DDSolver for G4, G5, and G7 but by DDSolver bootstrap for G5 only. In FaSSIF,



Figure 1. In vitro dissolution profiles of innovator and generic tablets in 0.5% SDS, FaSSIF, and FeSSIF. SDS: sodium dodecyl sulfate; FaSSIF: fasted state simulated intestinal fluid; FeSSIF: fed state simulated intestinal fluid.

12 Dissolution Technologies FEBRUARY 2024 www.dissolutiontech.com similarity was demonstrated by DDSolver for G1, G5, and G7, by DDSolver bootstrap for G7, and by Bootf2BCA and PhEq_bootstrap for G5 and G7. The only generic product that had an f_2 value higher than 50 according to all calculation methods was G7; only $\hat{f}_{2,bc}$ obtained from PhEq_bootstrap was not greater than 50 (Table 3).

Kinetic release parameters showed that the innovator MS tablet fit the Gompertz 1, Probit 1, and Weibull 2 models for 0.5% SDS, FaSSIF, and FeSSIF, respectively. The generic MS tablet parameters also fit different models in each media, with most of them fitting the Gompertz and Weibull models (Table 4).

Tablet Characterization

Tablet shapes or colors differed. Four of the seven generic products were square and similar to the innovator product, and three were round. All tablets were light pink except G4, which was yellowish. Diameter and thickness SD values were 5% or less except the innovator and G7 (6%) and G1 and G4 (7%).

According to the USP, the weight can exceed the limit of 7.5% deviation for a maximum of two out of 20 tablets (10%), but none can exceed 15% deviation from the average weight. For G2, G4, and G7, one tablet each exceeded the 15% deviation limit. Tablet weights of the other products were within the acceptable limit.

For content uniformity, the acceptable limit is within 15% of the label claim. All products were within this limit. Therefore, the out-of-limit tablet weights for G4 and G7 did not adversely affected content uniformity. Content uniformity must be assessed to ensure dosing accuracy in tablets with an active substance content below 25% or 25 mg.

Hardness values of the innovator and all generic products were higher than 50 N; however, a linear relationship between the tablet hardness and disintegration time could not be established. For example, despite G2 having the lowest tablet hardness, its disintegration time was the highest.

Table 1. Similarity Assessment by f_2 Bootstrap Method with DDSolver

Comparison	Observed Similarity Factor (f ₂)	Bootstrap (Mean)	5000 Bootstrap (5 th percentile)	5000 Bootstrap (95 th percentile)	Similarity Assessment
0.5% SDS					
I-G1	32.7	32.8	29.2	36.9	No
I-G2	23.7	23.6	21.5	25.6	No
I-G3	26.6	26.7	23.0	30.5	No
I-G4	48.3	49.7	36.3	68.9	No
I-G5	68.1	63.6	53.6	73.7	Yes
I-G6	24.1	24.1	21.7	26.7	No
I-G7	63.0	57.3	40.1	78.1	No
FaSSIF					
I-G1	58.0	54.6	43.0	70.9	No
I-G2	36.5 36.2 33.8		33.8	39.0	No
I-G3	28.5	28.2	26.2	30.4	No
I-G4	26.6	26.4	23.6	29.2	No
I-G5	66.5	59.5	49.6	67.7	No
I-G6	41.5	41.0	34.8	47.1	No
I-G7	72.1	63.4	51.0	77.0	Yes
FeSSIF					
I-G1	30.5	32.1	21.4	46.1	No
I-G2	25.4	25.4	22.1	28.8	No
I-G3	35.7	35.6	29.8	41.9	No
I-G4	62.1	61.3	51.3	72.2	Yes
I-G5	29.6	29.8	25.5	35.0	No
I-G6	30.3	30.1	18.5	46.0	No
I-G7	42.0	41.8	36.8	49.0	No

I: innovator, G: generic (G1–G7); SDS: sodium dodecyl sulfate; FaSSIF: fasted state simulated intestinal fluid; FeSSIF: fed state simulated intestinal fluid.

Table 2.	Similarity	Assessn	nent by f	2 Bootstr	ap Meth	od with E	Bootf2BC	A							_
		6	1	G	62	G	i3	6	14	G	5*		6	G	7*
	1			1		1	0.5%	SDS		1		1		1	
f ₂	CI Type	f ₂ : 3	32.7	f ₂ : 2	21.4	f ₂ : 2	24.0	f ₂ : 4	43.6	f_2	:-	f ₂ :	21.1	f_2	:-
Туре	- //	L	U	L	U	L	U	L	U	L	U	L	U	L	U
	Normal	28.7	36.8	18.4	21.2	18.9	27.5	43.0	63.3	-	-	18.2	23.8	-	-
, fa	Basic	29.0	36.3	18.7	21.1	19.7	27.0	44.1	65.5	-	-	18.2	23.5	-	<u> </u>
, , ,	PI	29.2	36.5	20.1	22.5	20.3	27.6	31.3	52.7	-	-	18.5	23.8	-	-
	Вса	27.3	36.2	19.8	21.9	20.0	27.1	39.1	53.3	-	-	18.7	24.9	-	-
		f ₂ : 3	32.5	f ₂ : 2	21.3	f ₂ : 2	23.7	f ₂ : 4	40.5	f ₂	:-	f ₂ :	21.0	f2	:-
	Normal	28.2	36.4	18.1	21.0	18.6	26.7	40.6	55.0	-	-	18.1	23.6	-	-
, farm	Basic	28.4	35.8	18.3	20.8	19.3	26.1	42.2	57.0	-	-	18.1	23.3	-	-
J 2,exp	PI	29.0	36.4	20.0	22.5	20.2	27.0	31.3	46.1	-	-	18.5	23.7	-	-
	Вса	27.2	36.1	19.7	21.7	20.0	26.5	35.9	46.6	-	-	18.7	24.9	-	-
							Fas	SIF							
f2	CI Type	f ₂ : !	54.7	f ₂ : 5	36.3	f ₂ :2	28.3	f ₂ : 2	26.5	f ₂ : !	59.6	f ₂ :	41.0	f ₂ : (53.5
Туре	спуре	L	U	L	U	L	U	L	U	L	U	L	U	L	U
	Normal	47.6	75.1	34.2	39.3	26.5	30.8	24.0	29.5	63.7	83.4	35.7	48.2	67.0	94.6
, Â	Basic	45.2	73.2	34.1	39.3	26.5	30.8	24.0	29.6	65.4	83.5	35.8	48.4	67.2	94.4
J 2	PI	42.8	70.9	33.8	39.0	26.2	30.4	23.7	29.2	49.6	67.7	34.6	47.1	49.9	77.1
	Вса	47.4	78.3	34.1	39.3	26.4	31.0	23.7	29.2	60.9	71.2	34.3	47.1	61.3	79.3
		f ₂ : 4	48.9	f ₂ : 3	35.9	f ₂ : 2	28.0	f ₂ : 2	26.2	f ₂ : !	53.5	f ₂ :	39.9	f ₂ : !	56.0
	Normal	43.3	57.9	33.5	38.6	26.0	30.2	23.5	29.0	52.5	61.4	33.9	45.6	50.0	66.9
â	Basic	43.3	58.1	33.3	38.4	25.9	30.2	23.4	28.9	53.4	61.8	33.9	45.3	49.8	65.3
J 2,exp	PI	41.4	56.2	33.5	38.6	25.9	30.2	23.5	29.0	48.7	57.1	34.3	45.7	49.1	64.7
	Вса	42.6	57.0	33.6	38.7	26.3	30.7	23.4	29.0	51.6	60.1	33.1	45.3	49.3	66.5
						1	Fes	SIF							
f ₂		f ₂ : 3	32.1	f ₂ : 2	25.5	f ₂ : 3	35.7	f ₂ : (51 .3	f ₂ : 2	29.8	f ₂ :	30.1	f ₂ : 4	41.8
Туре	спуре	L	U	L	U	L	U	L	U	L	U	L	U	L	U
	Normal	13.5	44.5	21.9	28.8	29.4	42.3	51.9	74.1	24.3	34.7	13.8	42.6	35.9	48.3
, Â	Basic	15.0	39.7	22.0	28.7	29.3	41.7	52.0	73.0	23.8	33.7	11.8	39.8	34.9	47.5
J 2	PI	21.4	46.1	22.1	28.8	29.8	42.2	51.3	72.3	25.5	35.4	18.4	46.5	36.5	49.0
	Вса	21.7	59.1	21.4	28.4	30.1	42.9	53.4	78.7	25.6	36.8	18.7	46.5	37.3	50.3
		f ₂ : 3	30.3	f ₂ : 2	25.3	f ₂ : 5	34.8	<i>f</i> ₂ : !	58.5	f ₂ : 7	29.5	f ₂ :	27.4	f ₂ : 4	41.1
	Normal	12.9	41.0	21.6	28.5	28.5	40.4	49.6	66.6	23.9	33.9	12.9	37.0	35.0	46.5
, f.	Basic	15.6	36.0	21.6	28.3	28.6	39.6	49.4	66.3	23.5	33.0	11.7	34.2	34.2	45.4
J 2,exp	PI	21.2	41.7	22.0	28.7	29.6	40.7	50.3	67.2	25.4	34.9	18.2	40.7	36.4	47.6
	Вса	21.5	46.1	21.1	28.2	29.6	40.7	50.3	66.9	25.5	36.5	18.2	40.7	36.6	48.0

*Could not be calculated.

^π_{2,exp}: expected similarity factor; CI: confidence interval; G: generic product (G1–G7); L: lower limit of 90% CI; U: upper limit of the 90% CI; PI: percentile; BCa: bias-corrected and accelerated; SDS: sodium dodecyl sulfate; FaSSIF: fasted state simulated intestinal fluid; FeSSIF: fed state simulated intestinal fluid.

DISCUSSION

Compared to the innovator product, f_1 and f_2 for generic products are expected to be less than 15% and greater than 50%, respectively. The results were within the limit values for G4, G5, and G7 in 0.5% SDS; G1, G5, and G7 in FaSSIF; and G4 in FeSSIF. Thus, none of the generic tablets had similar dissolution profiles with the innovator in all three media. There are studies showing that the oral bioavailability of montelukast is affected by food (5); however, the FDA recommends that in vivo bioequivalence studies can be performed under fasting or fed conditions, and the product monograph states that the product can be taken with or without food (12). Therefore, the observed variability may be due to the pH-dependent dissolution of montelukast, differences in formulation ingredients and production methods, tablet Table 3. Similarity Assessment by f_2 Bootstrap Method with PhEq_bootstrap

Product	f	2	$\hat{f}_{2,\text{average}}$	$\hat{f}_{2,bc}$	$\hat{f}_{2,exp}$
	L U				
		0.5%	SDS		
G1	29.0	32.4			
G2	20.0	22.5	21.4	21.5	21.2
G3	20.2	27.0	24.1	24.4	23.7
G4	35.5	63.9	51.0	46.8	46.1
G5*	-	-	-	-	-
G6	18.6	23.7	21.1	21.2	21.0
G7	36.2	48.9	53.6	13.1	41.9
		Fas	SIF		
G1	41.4	56.2	54.8	38.8	48.9
G2	33.5	38.6	36.2	36.6	35.9
G3	25.9	32.2	28.2	28.5	27.9
G4	23.4	29.0	26.4	26.7	26.2
G5	48.4	57.1	59.3	38.7	53.3
G6	33.8	45.6	40.9	42.2	39.8
G7	49.1	66.5	63.7	41.2	56.2
		FeS	SIF		
G1	21.5	41.7	32.4	35.1	30.5
G2	22.0	28.7	25.4	25.6	25.3
G3	29.6	40.7	35.5	36.4	34.7
G4	50.6	67.2	61.4	62.6	58.5
G5	25.4	34.9	29.8	30.2	29.5
G6	18.2	39.9	29.8	33.9	27.1
G7	36.5	47.2	41.7	42.6	41.0

*Could not be calculated.

 $f_{2,bc}$: bias-corrected $f_{2'}$: $f_{2,exp}$: expected $f_{2'}$: G: generic product (G1–G7); L: lower limit of 90% confidence interval (Cl); U: upper limit of the 90% Cl; SDS: sodium dodecyl sulfate; FaSSIF: fasted state simulated intestinal fluid; FeSSIF: fed state simulated intestinal fluid.

shape, the presence of surfactants that increase solubility at different rates, and/or different viscosities and buffering capacity of the media (4, 16). However, these differences do not have an exact equivalent in vivo because of the inappropriate dissolution method (17). For example, Prieto-Escolar et al. observed that the dissolution profiles of two film-coated tablets containing montelukast were similar, but they were not bioequivalent in vivo, so a new dissolution method was developed to establish the in vitro-in vivo correlation (IVIVC) (4).

Dissolution profiles of all generic tablets showed high variability at the early time points. This variability may be associated with variations in tablet placement and tablet-to-tablet variability (*18*). This situation may prohibit observation of the effect of formulation or manufacturing changes on drug release properties and create a major handicap in generic product development

(19). The similarity factor analysis is insufficient for statistical comparison of dissolution profiles because it does not contain a mathematical formula for statistical distribution in the calculation of f_2 (20). Moreover, it is difficult to evaluate type I (consumer's risk) and type II (manufacturer's risk) errors because f_2 is insensitive to the shape of the profiles (21). Therefore, regulatory authorities may recommend using an alternative statistical method, such as a 90% CI of f_2 based on the bootstrap methodology to compare dissolution profiles (7, 9). For example, Health Canada and the US FDA suggested the use of the \hat{f}_2 and BCa range (22). Compared to f_2 , the bootstrap-based f_2 approach is more sensitive in comparing dissolution profiles and is especially important when f_2 is less than 60. Among these approaches, Bootf2BCA and PhEq_bootstrap methods are based on a bootstrap percentile, and lower and upper limits are used. To support similarity, both limits should be above the cutoff value (\geq 50). On the other hand, DDSolver is solely based on the lower bound of the CI of bootstrap f_2 , and it is not recommended for comparing dissolution profiles with high variability as it cannot calculate parameters such as $\hat{f}_{2,\text{exp}}$ and $\hat{f}_{2,\text{bc}}$ (7). Because $\hat{f}_{2,\text{exp}}$ is the most prudent unbiased estimate of f_2 and is always defined, it should be used to conclude about the similarity of highly variable dissolution profiles (8). In the current study, f_2 was calculation with all approaches, and the bootstrap approach was more sensitive to detect similarity. Three tablets (G4, G5 and G7) were similar to the innovator in 0.5% SDS according to f_2 , but only one tablet (G4) was similar in the bootstrap approaches. When the f_2 values for the G4 in FeSSIF media were calculated with all methods (f_2 , $f_{2,exp}$, $f_{2,bc}$), similarity was higher than 50. Evaluation could not be performed for G5 and G7 with Bootf2BCA and for G5 with PhEq bootstrap in 0.5% SDS because the last two time points in the dissolution study were mathematically higher at the 20th minute than at the 30th minute.

When choosing the most suitable model and comparing models with different numbers of parameters, R^2_{adj} should be used instead of the coefficient of determination (R^2). R^2 will always increase as more parameters are included, whereas R^2_{adj} may decrease during the model fit. Therefore, the best model should be the one with the highest R^2_{adj} rather than R^2 (23). AIC is a parameter that depends on the size of the data and the number of data points. If the two models have a different number of parameters, it can be said that the model with the lower AIC value is better (24). MSC is a criterion for choosing a statistical model. MSC is modified from AIC and normalized to be independent of the scaling of data

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	Model parameters	l Gompertz 1	G1 Gompertz 2	G2 Gompertz 2	G3 Gompertz 2	G4 Gompertz 2	G5 Peppas- Sahlin 1	G6 Logistic 2	G7 Logistic 2
0.5% SDS		α: 2.10	α: 16.6	α: 67.9	α: 41.5	α: 3.61	<i>k</i> ₁ : 51.0	α: -6.53	α: -1.63
		β: 2.69	β: 3.93	β: 4.91	β: 4.64	β: 2.86	k ₂ : -6.93	β: 6.94	β: 3.15
			F _{max} : 88.9	F _{max} : 96.9	F _{max} : 95.3	F _{max} : 93.8	<i>m</i> : 0.43	F _{max} : 94.4	F _{max} : 98.5
	R ² adj	0.998	0.979	0.999	0.999	0.993	0.998	0.997	0.982
	AIC	14.0	26.1	14.3	11.2	20.4	14.1	18.2	26.2
	MSC	4.58	2.57	5.62	6.06	3.32	4.41	4.75	2.24
		Probit 1	Weibull 2	Quadratic	Weibull 3	Logistic 1	Logistic 1	Weibull 3	Korsmeyer- Peppas
FaSSIF		α: -1.19	α: 11.0	k ₁ : -0.001	α: 27.0	α: -2.69	α: -2.24	α: 12.1	k _{кР} : 29.8
		β: 1.51	β: 0.95	k ₂ : 0.052	β: 1.31	β: 1.94	β: 2.57	β: 1.04	n: 0.322
					F _{max} : 61.9			F _{max} : 115	
	R ² adj	1.00	1.00	0.992	0.998	0.999	0.995	0.993	1.00
	AIC	1.13	6.20	19.2	10.1	4.17	17.6	22.3	0.630
	MSC	7.20	6.58	3.69	5.26	5.96	3.98	3.72	7.30
		Weibull 2	Weibull 3	Logistic 1	Gompertz 2	Hixson- Crowell	Weibull 3	Weibull 4	Peppas- Sahlin 2
FeSSIF		α: 11.2	α: 6.93	α: -3.51	α: 44.0	<i>k</i> _{HC} : 0.022	α: 3.79	α: 27.1	<i>k</i> ₁ : 29.6
		β: 1.01	β: 0.885	β: 2.63	β: 4.44		β: 0.71	β: 1.38	k ₂ : -1.92
			F _{max} : 126		F _{max} : 125		F _{max} : 119	<i>T</i> i: 2.16	
								F _{max} : 69.3	
	R ² adj	0.999	0.999	1.00	0.993	0.999	0.998	0.999	0.995
	AIC	9.95	14.2	-4.52	24.2	9.77	17.1	6.76	19.9
	MSC	5.91	5.37	8.11	3.96	6.14	4.47	6.44	3.86

Table 4. Parameters for Mathematical Models and Descriptive Statistics for Dissolution of MS Innovator (I) and Generic (G1–G7) Tablets

 F_{max} : maximum fraction of drug released at infinite time; k_{KP} : release constant incorporating structural and geometric characteristics of drug-dosage form; m,n: diffusional exponent; Ti: location parameter that represents lag time; k_1 : constant related to Fickian kinetics and denotes relative contribution of $t^{0.5}$ -dependent drug diffusion to drug release; k_2 : constant related to Case-II relaxation kinetics and denotes relative contribution of t-dependent polymer relaxation to drug release in Peppas–Sahlin 2; Φ : standard normal distribution; α : scale factor; β : shape factor.

points. Among the different models, the model with the highest MSC value is the most suitable criterion. Considering these parameters, all generic tablets fit different dissolution models than the innovator, and most of them fit Gompertz and Weibull models. These results are similar to other studies, which reported that both Weibull and Gompertz frequently provide a good fit for different types of dissolution profiles (25). Considering the models that the generic tablets fit, it was determined that α (scale factor) and β (shape factors), which characterize the type of dissolution profile parameters, affected the dissolution behavior of MS. The effect of changes in these parameters on in vitro dissolution is evident. Therefore, no single method can be recommended as the best-fitting dissolution model, as others have pointed out (26). In addition, model-dependent methods have disadvantages such as the low number of time points for fast-dissolving immediate-release products and the fact that the most appropriate model selection is directly related to the product (22).

The shape of some generic tablets (G1, G3, and G4) differed from the innovator. The difference in tablet shape can affect both patient recognizability and in vitro dissolution results and may lead to errors in treatment (16). The observed weight deviation is related to poor powder flow properties. To improve powder flowability, granulation is performed, and lubricants are added to the formulation. Even though the same lubricant was used in the innovator and all generic products, high granule size or excessive use of lubricant may cause an increase in weight deviation. The diameter-thickness determination, which is an important parameter for packaging, is not a required test in the pharmacopeia. However, the general approach to evaluating the results is that the SD should not be more than 5% (27). Homogeneity of the coating influences deviations in diameter thickness of the filmcoated tablets, especially due to the tablet shape. Tablet hardness is also not a required test in pharmacopeia; however, it is stated in various sources that hardness values should be at least 50 N (5 kg) to have sufficient mechanical strength. On the other hand, if the tablet hardness is high, the disintegration time of the tablets may be delayed, which delays the onset of therapeutic effects especially for immediate-release tablets (28). All generic products had a longer disintegration time than the innovator. This may be because the disintegrant agents in the tablet formulations are different. Details of the production method of the innovator are confidential, but differences in production can affect tablet hardness, disintegration time, and dissolution (e.g., granule size, binder solution used during granulation, and the method of adding the disintegrant to the granule phase) (29).

A generic drug is the same as the innovator in terms of dosage form, administration route, and active ingredient, with similar efficacy, quality, and safety profiles within certain limits. However, generic products are produced by different companies and might contain different contents. According to Türkiye guidelines, for drugs/products that do not have a biowaiver, in vivo bioequivalence must be established (IVIVC), and comparative in vitro dissolution results should be presented with in vivo results. Additionally, the similarity of bioseries (series used for in vivo studies) should be demonstrated in vitro (30). However, recent studies have reported marked differences in therapeutic efficacy of marketed products containing the same amount of active ingredient, indicating that some generic products are not interchangeable with the innovator and/or each other (31). These differences may be because two-point dissolution analyses are considered sufficient instead of the full profile in batch-release studies, especially for immediate-release tablets, and the commercial lots are not analyzed despite the products being analyzed during the registration process (32, 33). Therefore, it is important to obtain a dissolution profile that demonstrates similarity with bioseries in batch-release studies.

In the current study, despite in vivo bioequivalence being established for the generic MS tablets in 0.5% SDS, similarity with the innovator product could not be established based on dissolution profiles using Bootf2BCA and PhEq_bootstrap methods. Possible reasons for the observed differences are that the dissolution medium might not be sufficient to show differences or similarities in the drug release profile, and/or the dissolution method (with USP apparatus 2) may not fully reflect the in vivo conditions for pH-dependent, poorly water-soluble, firstpass metabolism-exposed drugs such as MS (*34, 35*).

CONCLUSION

The similarity factor f_2 is still the most common and accepted tool for dissolution profile comparisons;

however, the bootstrap f_2 approach, which has a 90% Cl of expected f_2 , seems to be a more conservative way to assess the similarity of dissolution profiles, especially when the profiles show high variability. When comparing model-dependent and independent analysis methods, the dissolution profiles of generic products with proven in vivo bioequivalence in FDA-recommended media were different, especially in FaSSIF and FeSSIF media. The differences may be due to the weak acid structure of MS changing with the pH of media with a low buffer capacity, such as distilled water, or it may also be because the dissolution method cannot fully reflect the in vivo conditions due to the first-pass effect of MS. Therefore, dissolution methods should be developed that can better reflect in vivo conditions for pH-dependent and lowsoluble drugs such as MS. Moreover, the use of PhEq_ bootstrap and/or Bootf2BCA methods instead of the f_2 should be accepted by the guidelines.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article and may be requested by contacting the corresponding author.

ACKNOWLEDGMENT

The authors thank Mehmet Akif Oksuz (AssisTT) and Dogukan Oksuz (Havelsan) for their assistance in using R.

DISCLOSURES

This work was supported by the Karadeniz Technical University Scientific Research Projects Coordination Unit (THD-2021-9749) and the Suleyman Demirel University Scientific Research Projects Coordination Unit (TSG-2022-8785).

The authors have no conflicts of interest.

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Question & Answer Section

The following questions have been submitted by readers of Dissolution Technologies. Margareth R. Marques, Ph.D., and Mark Liddell, Ph.D., United States Pharmacopeia (USP), authored responses to each of the questions. *Note: These are opinions and interpretations of the authors and are not necessarily the official viewpoints of the USP. E-mail for correspondence: mrm@usp.org.

 $Q\,$ In the USP monograph for Esomeprazole Magnesium Delayed Release Capsules, Dissolution Test 1, there is not quantitation step in the acid stage. Are there any cases where this quantitation step can be skipped?

A The development of any dissolution test should always start by using the standard conditions. In the case of delayed release dosage forms, the development should use initially the conditions stated in the USP general chapter <711> Dissolution. The procedure for delayed-release dosage forms has two methods, Method A and Method B. It is up to the developers to decide which is most appropriate using a case-by-case approach for each new dosage form. Deviations from the standard procedure are allowed with scientific justification when supported by data obtained from the samples being evaluated.

$Q\,$ Can gelatin cross-linking occur either in hard or soft gelatin capsules?

A Yes, gelatin cross-linking can occur in any type of gelatin capsules as well as other dosage forms coated with gelatin. Gelatin, in the presence of certain compounds such as formaldehyde, or in high temperature/high humidity conditions, can form covalent bonding between gelatin chains that are irreversible. Proteolytic enzymes can break this bond. For more information, see USP general chapter <1094> Capsules – Dissolution Testing and Related Quality Attributes.

Q What is the reason for several USP monographs for dosage forms to have multiple dissolution tests? Which one of these tests should be used? Is it possible for an immediate-release dosage form monograph to have more than one dissolution test?

A The USP monograph may have multiple dissolution tests when a) a poorly soluble drug (BCS class 2 or 4; see USP general chapter <1090>) is formulated in an immediate release dosage form, or b) any drug substance is formulated in a modified-release dosage form (extended or delayed). Because different manufacturers use different formulation strategies to increase the drug solubility and/or to achieve the desired drug release profile, in both cases the dissolution test is likely to be formulation dependent. In addition, the dissolution test must also be discriminative for the critical quality attributes of the formulation. These critical quality attributes may also be dependent on the formulation manufacturing processes. The dissolution, disintegration, and/or drug release tests in USP monographs are the studies that have been approved by the US FDA for products marketed in the USA.

Q Should samples from the dissolution vessel be collected with the paddle or basket in motion or should it be stopped before sampling?

A During the dissolution test, the contents of the vessel are essentially a suspension, containing undissolved and dissolved drug particles and excipients. To obtain as homogeneous a sample as possible, the paddle or the basket must be in motion during the sampling process.

$Q\,$ How should dissolution results be expressed? If the tolerances are NLT 80% (Q), how should the results be rounded?

A Ingeneral, analytical results should always be rounded based on the acceptance criterion under consideration. If the results are going to be used for quality control, the values should be reported with the same number of significant figures as the acceptance criteria (see USP General Notices and Requirements). In contrast, when the dissolution results are used during product/method development, it may be more useful to consistently round the values to one or two decimal places to allow more precision when evaluating product performance.

Q When should one use a basket versus a paddle apparatus?

A Typically, baskets are used when the dosage form floats and/or sticks to the vessel wall. In general, when operated at the same rotation speed, the fluid velocities observed inside the vessel are, on average, lower with baskets when compared with the paddle apparatus. This leads to differences in the shear force distribution throughout the vessel. Consequently, the use of baskets may not be appropriate for some formulations even if the dosage form does float. Baskets are useful with certain dosage forms, such as osmotic pump tablets or films. Ultimately, the selection of the dissolution apparatus should be decided on a case-by-case basis and must be justified with results obtained from the samples being evaluated.

Q In the USP general chapter <1092> The Dissolution Procedure – Development and Validation, under section 5.3 Accuracy/Recovery, there is a reference to a case in which the sample has very low strength, which states that "it may be more appropriate to prepare a stock solution than to attempt to weigh very small amounts." Does this mean that one can prepare a drug substance stock solution for the entire accuracy study, and add appropriate aliquots to each vessel instead of weighing powder?

A The USP chapter <1092> contains recommendations that may be adapted for the individual dissolution procedure under development. It is up to the analytical scientist to select conditions that are the most suitable for each project. In practice, a standard stock solution could be used in any project. Depending on the solubility of the compound when diluted in the media and the effect of additional organic solvent on the analytical method, the stock solution may be prepared in organic solvent whenever appropriate. See <1092> for more details and examples.

 ${f Q}$ The USP general chapter <711> Dissolution has a note stating that "Where multiple sampling times are specified, replace the aliquots withdrawn for analysis

with equal volumes of fresh dissolution medium at 37 °C, or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test and verify the temperature of the mixture under test at suitable times." In the case of single point sampling, does the temperature need to be verified at suitable times?

A The temperature of the medium inside the vessel is checked before starting the test. The test can only be initiated if all the vessels equilibrate to the appropriate temperature, i.e., 37 ± 0.5 °C. Typically, the temperature is also measured at the end of the test to verify if the test was performed under the appropriated temperature conditions. The temperature of the dissolution medium inside the vessel is not measured during the dissolution test because the introduction of a thermometer or probe may modify the hydrodynamics within the vessel and could impact the dissolution results. Some dissolution test equipment includes temperature sensors within the paddle or basket shaft, and it is possible to monitor the temperature while the dissolution test is being performed without introducing and external temperature probe. In either case, when the dissolution instrument is gualified, the ability of the dissolution equipment to maintain temperature control is a key operational parameter that must be verified (see USP Guideline on Procedures for Mechanical Calibration and Performance Verification Test Apparatus 1 and Apparatus 2).



Every issue of *Dissolution Technologies* features a Question and Answer section. This section is designed to address general dissolution questions submitted by our readers.

Please send your questions to: Attn: Q&A

9 Yorkridge Trail, Hockessin, DE 19707 Email: vagray@rcn.com Submit via our website: www.dissolutiontech.com

Calendar of Events

February 20–23, 2024

Advanced GastroPlus®DMPK and Clinical

Pharmacology Workshop

Location: Online Registration: https://www.simulations-plus.com/events/ advanced-gastroplusr-dmpk-clinical-pharmacology-workshopvirtual/

February 22, 2024

Dissolution Discussion Group Quarterly Online Meeting—Expert insights on dissolution testing of generic pharmaceutical products

Location: DDG Online Meeting at 10:30 am ET Registration: https://www.agilent.com/chem/dissolutionwebinars

March 4–27, 2024

PBPK Modeling for FIH Predictions

Location: Online Registration: https://www.simulations-plus.com/events/pbpkmodeling-for-fih-predictions-virtual-workshop-2/

March 5, 2024

Complimentary Introduction to GastroPlus®

Workshop

Location: Online Registration: https://www.simulations-plus.com/events/ complimentary-introduction-to-gastroplus-workshop-15/

March 11-15, 2024

GastroPlus® Introductory Workshop- PDT

Location: Online Registration: https://www.simulations-plus.com/events/ gastroplus-introductory-workshop-pdt-virtual/

April 1-30, 2024

GastroPlus® Application-Based DDI Workshop Location: Online

Registration: https://www.simulations-plus.com/events/ gastroplus-application-based-ddi-workshop-2/

April 8, 2024

Complimentary Introduction to GastroPlus®

Workshop Location: Online Registration: https://www.simulations-plus.com/events/ complimentary-introduction-to-gastroplus-workshop-16/

April 22-25, 2024

GastroPlus® Advanced Workshop: Pharmaceutical

Development Location: Online

Registration: https://www.simulations-plus.com/events/ gastroplus-advanced-workshop-pharmaceuticaldevelopment-2/

May 23, 2024

Dissolution Discussion Group Quarterly Online Meeting—Key considerations for dissolution software and compliance

Location: DDG Online Meeting at 10:30 am ET Registration: https://www.agilent.com/chem/dissolutionwebinars

July 8-12, 2024

Controlled Release Society 2024 Annual Meeting

Location: Bologna, Italy For information, visit http://www.controlledreleasesociety.org/ meetings/annual



July 25, 2024

Dissolution Discussion Group Quarterly Online Meeting—Applications for predictive dissolution testing

Location: DDG Online Meeting at 10:30 am ET Registration: https://www.agilent.com/chem/dissolutionwebinars

October 20-23, 2024

PharmSci 360 AAPS Meeting

Location: Salt Palace Convention Center, Salt Lake City, UT, USA For information, visit https://www.aaps.org/pharmsci/annual-meeting

November 18-20, 2024

Eastern Analytical Symposium and Exhibition

Location: Crowne Plaza Princeton-Conference Center, Plainsboro, NJ, USA For information, visit eas.org

November 21, 2024

Dissolution Discussion Group Quarterly Online Meeting—Dissolution method development

guidance using QbD

Location: DDG Online Meeting at 10:30 am ET Registration: https://www.agilent.com/chem/dissolutionwebinars

On Demand Events

- Simplifying Dissolution Automation with In-Situ Flber Optic UV On Demand
 https://www.distekinc.com/watch/webinar-simplifyingdissolution-automation-with-in-situ-fiber-optic-uv/
- Clarifying 21 CFR Part 11 & Data Integrity Requirements for Dissolution Testing On Demand

www.distekinc.com/watch/clarifying-21-cfr-part-11-and-data-integrity-for-dissolution-testing/

- Ocular Administration (OCAT[™]) in GastroPlus[®] On Demand https://www.simulations-plus.com/events/gastroplus- additional-dosage-routes-workshop-ocular-administration-ocat-virtual/
- Oral Cavity Administration (OCCAT™) in GastroPlus® On Demand https://www.simulations-plus.com/events/gastroplusadditional-dosage-routes-workshop-oral-cavityadministration-occat-virtual/
- **Pulmonary Administration** (**PCAT™**) in GastroPlus[®] On Demand https://www.simulations-plus.com/events/gastroplusadditional-dosage-routes-workshop-pulmonaryadministration-pcat-virtual/
- GastroPlus[®] ADR 4 Course Bundle (TCAT[™] / OCAT[™] / OCCAT[™] / PCAT[™]) https://www.simulations-plus.com/events/gastroplus-adr-4-course-bundle-tcat-ocat-occat-pcat/
- GastroPlus[®] ADR 5 Course Bundle (TCAT[™] /OCAT[™]/OCCAT[™]/PCAT[™]/Injectables) https://www.simulations-plus.com/events/gastroplus-adr-5-course-bundle-tcat-occat-pcat-injectables/
- Transdermal Administration (TCAT™) in GastroPlus[®] https://www.simulations-plus.com/events/gastroplus-

https://www.simulations-plus.com/events/gastroplusadditional-dosage-routes-workshop-transdermaladministration-tcat-virtual/

 Injectables (IM, SQ, IA) in GastroPlus[®] Including Biologics and LAIs

https://www.simulations-plus.com/events/gastroplusadditional-dosage-routes-workshop-injectables-incl-laibiologics-virtual/





Logan Instruments Announces Dry Heat Pro Series Dissolution Tester

Logan Instruments Corp. is proud to announce the next evolution in dissolution apparatus 1, 2, 5, and 6

The **Dry Heat Pro Series Dissolution Tester** replaces the traditional water bath. Each vessel has three-zone, contemporary, dry heat elements. The unique design ensures there is no cold zone at the bottom of the vessel. The three dry heat elements can be selected for optimal heating of any volume from 100 to 1000 mL. Dry heating ensures more rapid heating of the vessel media than can be achieved with a water bath. Vessel wall sensors protect against thermal shock, so standard vessels can be used. The sensors also maintain accurate and consistent temperature throughout each vessel for the duration of the test.

The Dry Heat Pro Series Dissolution Tester is available in 8, 12, 15, or 18 vessel configurations. All models comply with the requirements for R&D and QC. The larger capacity systems allow multiple QC batches to be run simultaneously.

Logan offers these models with up to three optional infra-red cameras for each vessel. Infrared imaging allows the study to run in complete darkness, avoiding the adverse effect of light on the test compound. One camera beneath the vessel, one on the side, and a third inside the shaft. These images are recorded for subsequent review and can help explain anomalous results.



Another new introduction to these models is **in-line UV analysis with fiber optic probes**. UV can also be measured online by sampling through Logan's parallel, 8 flow-cell spectrophotometers. As with all Logan dissolution systems the samples can also be automatically collected for off-line analysis.

To further advance your research the new dissolution apparatus connects directly to Logan's PERMETRO to economically streamline bioequivalence studies.

For more information about the new generation Dry Heat Pro Series Dissolution Tester please visit http://www. loganinstruments.com or contact us at infoDT@loganinstruments.com





Simulations Plus Embarks on Collaboration with Northeastern University and The TIM Company Through New FDA Grant

Partnership aims to integrate experimental data and PBPK modeling to identify key formulation factors to accelerate modified-release product development

Lancaster, CA – Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation solutions for the pharmaceutical, biotechnology, chemicals, and consumer goods industries, today announced that, through a joint proposal with Northeastern University and The TIM Company, it has been awarded a new funded grant from the U.S. Food and Drug Administration (FDA). The project will aid in the understanding of oral modified-release (MR) formulations and advance the development and approval of generic oral MR drug products through the combination of novel in vitro testing and mechanistic modeling and simulation.

For this award, Dr. Jie Shen, Associate Professor of Pharmaceutical Sciences, and her lab at Northeastern University, along with partners at The TIM Company, will generate in vitro data characterizing the critical quality attributes (CQAs) and dissolution of multiple strengths of MR formulations. The newly generated data will be used to parameterize physiologically based pharmacokinetic/physiologically based biopharmaceutic (PBPK/PBBM) models predicting clinical pharmacokinetics of those MR formulations following their oral administration. The combination of in vitro and in silico studies will support the identification of the appropriate factors to scale the MR formulation for additional strengths, and to identify the CQAs and formulation design spaces for oral MR tablets.

"This collaboration is an exciting step forward, as we combine our PBPK/PBBM modeling and simulation expertise with Northeastern University's research excellence and The TIM Company's groundbreaking tiny-TIMsg model," said Dr. Xavier Pepin, Associate Vice President, Regulatory Strategies and lead investigator for this grant for Simulations Plus. "Together, we are charting a course towards more efficient drug development and safer healthcare solutions. We believe the comprehensive framework established through this collaboration will have value for both the FDA and the companies involved in developing oral MR formulations."

"The research efforts of our team and Dr. Shen have the potential to accelerate the availability of critical treatments to patients across the world, with minimized need for clinical trials," said Susann Bellmann, Chief Technology Officer at The TIM Company. "We are proud to support those efforts and this collaboration through the use of our dynamic in vitro gastrointestinal model, tiny-TIMsg."

FDA scientific and program staff will actively collaborate with Northeastern University, The TIM Company, and Simulations Plus. Dr. Pepin, with assistance from Dr. Maxime Le Merdy at Simulations Plus, will oversee the modeling and simulation activities of the contract.

Funding for this collaboration is made possible by the Food and Drug Administration through grant award 1U01FD007959-01. Views expressed in this press release do not necessarily reflect the official policies of the Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.



Simplifying Dissolution Testing: Opt-Diss UV Fiber Optic System Software Rev. 3.20 Delivers Streamlined Processes and Expanded Capabilities

North Brunswick, NJ – Distek, Inc., a well-reputed leader in laboratory pharmaceutical instruments, is excited to announce the release of the latest software update for the Opt-Diss - In-Situ Fiber Optic UV System for Dissolution Testing: Version 3.20. This update introduces significant enhancements designed to streamline the dissolution testing process and expand the system's capabilities.

KEY UPDATES IN VERSION 3.20

Integrated Media Change/Addition: Analyzing tests employing changing or adding additional media during testing has been greatly simplified. Previously, for complex dosage forms like enteric-coated tablets, users had to run two separate methods and generate combined reports using third-party software. The new version automates this process. It introduces the use of pre- and post-media change blanks, automatically pauses measurements for media changes, and generates a consolidated report with visual demarcation of media change instances.

Dosage Weight Correction Feature: This update allows for recording individual dosage weights, enabling the correction of % dissolved/concentration values for each dosage form. This feature is particularly beneficial for non-uniform dosage forms such as hand-pressed tablets, powder samples, and medical devices in early-stage research.

Run Automation for Complete Systems: For systems comprised of Distek baths equipped with a Dosage Auto-Dropper (DDS) option, complete run automation is now possible. Technicians can initiate the process with a simple start command. The software autonomously manages agitation and heating, dosage dropping at optimal media temperatures, and data collection, culminating in the automatic generation, storage, and optionally printing of final reports.

ENHANCED USABILITY AND EFFICIENCY

These updates significantly enhance the Opt-Diss functionality and user-friendliness, reinforcing its status as a vital tool in pharmaceutical and medical research and quality control laboratories. Our commitment to continuous innovation and improvement aims to meet the evolving needs of our users.

For more information or to upgrade to Version 3.20, contact our sales support team at sales@distekinc.com.







Cipher

21 CFR Part 11 Instrument Control Software

- Complete 21 CFR Part 11 and Data Integrity compliance for Distek dissolution, autosamplers, and disintegration instruments.
- Method Wizards simplify method creation.
- Remotely configure and monitor Distek instruments in real-time from your PC.
- Enable automatic export of record files for seamless integration with a LIMS package.







Schedule your Free Cipher Demonstration







Elevating the Dissolution Environment

The Agilent 280-DS Mechanical Qualification System (MQS) enables the physical qualification of USP dissolution Apparatus 1 (Rotating Basket) and 2 (Rotating Paddles) using Enhanced Mechanical Qualification (EMQ) guidelines. The system's sensing technology allows hands-free measurements to be performed in seconds, while recording critical physical parameters.

A proactive approach. Easily shorten your qualification interval for more frequent insight into instrument performance, reducing the chance of failures.

Save time. Instant feedback helps the user investigate aberrant results or abnormalities at an early stage and reduce errors.



For more information about the Agilent 280-DS, visit: www.agilent.com/chem/280-DS

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